Circulating Tumour Cells in Cancer- Pathway to Precision Oncology

Prof Colleen Coyne Nelson
Executive Director, Australian Prostate Cancer Research Centre - Queensland
Professor and Chair Prostate Cancer, Institute of Health and Biomedical Innovation, Queensland University of Technology
Translational Research Institute
Circulating tumor cells (CTCs) were first described in 1869 by Dr Thomas Ashworth, an Australian physician. When observing under the microscope the blood of a man with metastatic cancer, he discovered “cells identical with those of the cancer itself” and postulated that they “may tend to throw some light upon the mode of origin of multiple tumors existing in the same person.”

THE DISSECTING ROOM IN 1864 University of Melbourne. SHOWING HALFORD WITH FIRST AND SECOND YEAR STUDENTS/FROM LEFT TO RIGHT:/OCTAVIUS VERNON LAWRENCE, THOMAS RAMSDEN ASHWORTH, PATRICK MALONEY, FRANCIS LONG, ALEXANDER MACKIE, DR. GERALD HARRY FEATHERSTONE, PROFESSOR GEORGE BRITTON HALFORD, WILLIAM CAREY REES./DR. FETHERSTONE ACTED AS AN UNOFFICIAL PROSECTOR TO PROFESSOR HALFORD./IN THE BACKGROUND IS THE MEDICAL SCHOOL PORTER.'


Patients succumb to cancer progression to metastases and treatment resistance. Can CTCs offer an ‘liquid biopsy’ of metastatic cells for prognostication and inform treatment decisions?
Epithelial cell/CTC
EpCam+, CK+, DAPI+

Leukocyte
EpCam-, CK-, DAPI+ CD45+

CellSearch CTC Identification

CellSearch Circulating Tumor Cell Test

Nucleus
DAPI

EpCam

Leukocyte
EpCam-

CD45

Cyto keratin

Ferromagnetic
Significance of Circulating Tumor Cells Detected by the CellSearch System in Patients with Metastatic Breast Colorectal and Prostate Cancer

References:
CELLSEARCH® Circulating Tumor Cell Kit (Epithelial) Instructions for Use. Veridex, LLC
CTC Enumeration >5 in 7.5 ml blood by CellSearch prognostic for survival

Circulating Tumor Cells: A Novel Prognostic Factor for Newly Diagnosed Metastatic Breast Cancer
Massimo Cristofanilli, et al. JCO vol 23, p1421. 2005

Circulating Tumor Cells Predict Survival Benefit from Treatment in Metastatic Castration-Resistant Prostate Cancer

Significance of Circulating Tumor Cells Detected by the CellSearch System in Patients with Metastatic Breast Colorectal and Prostate Cancer
M. Craig Mills,*, Gerold V. Doyle,*, and Leon W. M. M. Terstappen*
CTC Enumeration Remain or Convert to favourable following treatment within 3-5 weeks

<5CTCs prognostic for survival

**Breast**

- **Remain favorable**: 83 (47%)
- **Convert to unfavorable**: 38 (21%)
- **Convert to favorable**: 39 (22%)
- **Remain unfavorable**: 17 (10%)

**Prostate**

- **Remain favorable**: 45 (20%)
- **Convert to unfavorable**: 88 (38%)
- **Convert to favorable**: 71 (31%)
- **Remain unfavorable**: 26 (11%)

**Colon**

- **Remain favorable**: 303 (70%)
- **Convert to unfavorable**: 74 (17%)
- **Convert to favorable**: 24 (6%)
- **Remain unfavorable**: 29 (7%)

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**Review Article**

*Significance of Circulating Tumor Cells Detected by the CellSearch System in Patients with Metastatic Breast Colorectal and Prostate Cancer*


M. Craig Miller, Gerald V. Doyle, and Loen W. M. Terstappen

*Circulating Tumor Cells Predict Survival Benefit from Treatment in Metastatic Castration-Resistant Prostate Cancer*

Johann S. de Bono, Howard I. Scher, R. Bruce Montgomery, Christopher Parker, M. Craig Miller, Henk Tilting, Gerald V. Doyle, Loen W. M. Terstappen, Kenneth J. Pinta, and Derek Raghavan

CTC Enumeration dynamics predicts treatment response in metastatic colorectal cancer and augments imaging for classification of progressive disease

Not all CTCs may be equals: characterised by physical and biological properties.
Capturing CTCs Physical and Biological approaches

Antibody
- EpCam
- Cell surface antigens
- Tumour specific

Cellular assays

Microfluidics
Microfiltration
Density centrifugation

Circulating Tumor Cells and Circulating Tumor DNA

Catherine Alix-Panabières,1,2,3
Heidi Schwarzenbach,4 and Klaus Pante1

Microfiltration for CTC capture

Clinical Translation of a Novel Microfilter Technology
Capture, Characterization and Culture of Circulating Tumor Cells

Anant Khadse, Srikumar Rout, Jiaojiao Sun, Tien-Tzong Pan, Mathjsumolu, Minghua Zhou, Sheng Zhang, Yu-Yi Chuang, Ted, Randal E. Gil, and Ram Das,1
1Center for Nanomedicine, University of Maryland, College Park, Maryland 20742, USA

Fig. 1. (A) 2-D microfilter wafer. (B) Close-up view 2-D microfilter with circular pores. (C) 3-D microfilter for live cell capture

Fig. 2. (A) CTC (CK=CD45+) and PSMC (CK=CD45+) (B) scanning on filter. CK=Alexa 488 (green) and CD45=Alexa 594 (red). (B) DNA FISH for HER2. (C) Morphological characteristics were in CTC from different cancers

Critical Review
Enrichment, Detection and Clinical Significance of Circulating Tumor Cell
Sunil Kumar Arya, Bing Lim and Abdur Rub Abdur Rahman
Lab Chip, 2013, Accepted Manuscript

DOI: 10.1039/C3LC00009E
Received 03 Jan 2013, Accepted 18 Mar 2013
Combine Filtration and Microfluidics for CTC capture

**A**

Filtration

**B**

MCF-7 breast cancer cells

A *Nano-Valero* device captures circulating tumor cells and keeps them viable for further analysis.

*Lab Chip, 2012, 12, 1753–1767*
Micro-Centrifugation for CTC capture

Isolation and retrieval of circulating tumor cells using centrifugal forces
Han Wei Hou, Majid Ebrahimi Warkiani, Bee Luan Khoo, Zi Rui Li, Ross A. Soo, Daniel Shao-Weng Tan, Wan-Teck Lim, Jongyoon Han, Ali Asgar S. Bhagat, & Chwee Teck Lim
Scientific Reports 3, Article number: 1259 doi:10.1038/srep01259 Received 02 October 2012 Accepted 28 January 2013 Published 12 February 2013
Circulating Tumor Cells
A New Opportunity for Therapeutic Management of Cancer Patients
By Martin Fleisher, PhD, FACB
November 2008 Clinical Laboratory News

On-Q-ity is commercializing a microfluidic approach developed at MGH for capturing rare circulating tumor cells for quantification and analysis. Image credit: Lecia V. Sequist, Sunitha Nagrath, Mehmet Toner, Daniel A. Haber, and Thomas J. Lynch


Figure2 Examples of CTC capture technologies that combine multiple enrichment principles. A: The DEP field-flow fractionation device developed by Gascoyne and colleagues combines the use of hydrodynamic l...

Microfluidics and Circulating Tumor Cells
Yi Dong, Alison M. Skelley, Keith D. Merdek, Kam M. Sprott, Chunsheng Jiang, William E. Pierceall, Jessie L...
DEPArray System is a microelectronic active silicon substrate embedding control circuitry for addressing each individual dielectrophoretic (DEP) cage.
A novel method for the *in vivo* isolation of circulating tumor cells from peripheral blood of cancer patients using a functionalized and structured medical wire

NADIA SAUCEDO ZENI¹, STEFFI MEWES¹, ROBERT NIESCH¹, LAUKASZ GASIOROWSKI², DAVID MURAWA³, PIOTR NOWACZYK², TATIANA TOMASZ¹, EKKEHARD WEBER⁴, GRZEGORZ DWORACKI², NILS G. MORGENTHALER¹, HEIKE JANSEN⁴, CORINNA PROPPING⁵, KAROLINA STERZYNSKA⁶, WOJCIECH DYSZKIEWICZ⁷, MACIEJ ZABEŁ⁸, MARION KIECHE⁴, UTE REUNING⁴, MANFRED SCHMITT⁴ and KLAUS LUCKE¹

¹GILLUPi GmbH, Potsdam, Germany; ²Department of Thoracic Surgery, WCP11: Poznan University of Medical Science; ³First Department of Surgical Oncology and General Surgery, Wielkopolska Cancer Center, 61-866 Poznan, Poland; ⁴Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; ⁵Poznan University of Medical Science, Fakultas 10, 61-701 Poznan, Poland

Received February 17, 2012; Accepted April 3, 2012

Figure 1. Schematic drawing of the functionalized tip of the FSMW. Antibodies to the epithelial cell surface antigen EpCAM are attached to a polycarboxylate hydrogel (1-3 μm) which is coated on a gold-plated (200 μm) Soldinger guidewire. Then the hydrogel is functionalized with antibodies to the EpCAM. This FSMW interacts with target cells expressing EpCAM antigen on their surface, e.g., CTC of breast and lung cancer patients.
Application of CTC capture and analysis throughout cancer management.

<table>
<thead>
<tr>
<th>Patient Blood</th>
<th>CTC Isolation</th>
<th>CTCs</th>
<th>Applications</th>
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<tr>
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<td>Immunostain</td>
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<td>Cell culture</td>
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Yu M et al. J Cell Biol 2011;192:373-382
Growing array of new targeted oncology drugs matched to specific mutations or aberrant biological pathways

Promising molecular targeted therapies in breast cancer

Radha Munagala¹, Farrukh Aqil¹, Ramesh C Gupta²

REVIEW ARTICLE
Year : 2011 | Volume : 43 | Issue : 3 | Page : 236-245
Growing array of new targeted oncology drugs matched to specific mutations or aberrant biological pathways

<table>
<thead>
<tr>
<th>Drug</th>
<th>Year Approved</th>
<th>Tumor Type</th>
<th>Biomarker</th>
<th>Linked to Indication and Usage</th>
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<tr>
<td>Trisenox® arsenic trioxide</td>
<td>2000</td>
<td>Leukemia</td>
<td>PML/RARA</td>
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<td>Ad cetin® bortezomib vedotin</td>
<td>2011</td>
<td>Lymphoma</td>
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<td>Myleran® busulfan</td>
<td>1954</td>
<td>Leukemia</td>
<td>Philadelphia chromosome</td>
<td>No</td>
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<td>Xeloda® capcitabine</td>
<td>1998</td>
<td>Colorectal cancer</td>
<td>DPD</td>
<td>No</td>
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<td>Erbitux® cetuximab</td>
<td>2004</td>
<td>Breast cancer</td>
<td>EGFR, KRAS</td>
<td>Yes</td>
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<td>Platino® cis-platin</td>
<td>1978</td>
<td>Multiple solid tumors</td>
<td>TPMT</td>
<td>No</td>
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<tr>
<td>Xalostar® cetuximab</td>
<td>2011</td>
<td>NSCLC</td>
<td>EMLA-ALK</td>
<td>Yes</td>
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<td>Sipulevax® dasatinib</td>
<td>2006</td>
<td>Leukemia</td>
<td>Philadelphia chromosome</td>
<td>Yes</td>
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<td>Onxkyx® denileukin ditox</td>
<td>1999</td>
<td>Lymphoma</td>
<td>CD25</td>
<td>Yes</td>
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<td>TargeT® erlotinib</td>
<td>2004</td>
<td>Colorectal cancer</td>
<td>EGFR</td>
<td>No</td>
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<td>Alimta® theralim</td>
<td>2009</td>
<td>RCC</td>
<td>Her2/neu</td>
<td>Yes</td>
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<td>Aromasin® exemestane</td>
<td>1999</td>
<td>Breast cancer</td>
<td>ER and PGR</td>
<td>Yes</td>
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<td>Adrucil®, Cerase®</td>
<td>1962</td>
<td>Multiple solid tumors</td>
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<td>No</td>
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<td>Eluxet® S-5 fluoruracil</td>
<td>2002</td>
<td>Breast cancer</td>
<td>ER</td>
<td>Yes</td>
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<td>Faslodex® fulvestrant</td>
<td>2003</td>
<td>NSCLC</td>
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<td>Inexa® geltimib</td>
<td>2003</td>
<td>Leukemia</td>
<td>PDGFR, c-kit, Philadelphia chromosome</td>
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<td>Gleevec® imatinib</td>
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<td>Campotar® irinotecan</td>
<td>1996</td>
<td>Colorectal cancer</td>
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<td>Tykerb® lapatinib</td>
<td>2007</td>
<td>Breast cancer</td>
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<td>Revlimid® lenalidomide</td>
<td>2005</td>
<td>Multiple myeloma</td>
<td>Chromosome 5q</td>
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<td>Femara® letrazole</td>
<td>1997</td>
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<td>Purinol® mercaptopurine</td>
<td>1953</td>
<td>Leukemia</td>
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<td>Tarceva® nilotinib</td>
<td>2007</td>
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<td>Philadelphia chromosome</td>
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<td>Vectibix® panitumumab</td>
<td>2006</td>
<td>Colorectal cancer</td>
<td>EGFR, KRAS</td>
<td>Yes</td>
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<td>Pertuzumab® pertuzumab</td>
<td>2012</td>
<td>Breast cancer</td>
<td>Her2/neu</td>
<td>Yes</td>
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<td>Eribiti® erasunib</td>
<td>2002</td>
<td>Leukemia, lymphoma, solid tumors</td>
<td>Her2/neu</td>
<td>Yes</td>
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<td>Novaldi® tamoxifen</td>
<td>1977</td>
<td>Breast cancer</td>
<td>ER</td>
<td>Yes</td>
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<td>Tabloid® thiguanine</td>
<td>1966</td>
<td>Leukemia, lymphoma, solid tumors</td>
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<td>Nexar® ixotumomab</td>
<td>2004</td>
<td>Lymphoma</td>
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<td>Horzono® naxanumab</td>
<td>1998</td>
<td>Breast cancer</td>
<td>Her2/neu</td>
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<td>Zelboraf® vemurafenib</td>
<td>2011</td>
<td>Melanoma</td>
<td>BRAF</td>
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</table>

Source: U.S. Food and Drug Administration website. Table of Pharmacogenetic Biomarkers in Drug Labels
http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm?utm_campaign=Google2&utm_source=fdaSearch&utm_medium=website&utm_term=biomarkers&utm_content=1
How do we analyse and what does it mean—the challenges of tumour cell heterogeneity and dynamics of cancer progression

Pooled Cells or fDNA analysis

Individual cells given tumour heterogeneity

Circulating Tumor Cells and Circulating Tumor DNA

Catherine Alix-Panabères,1,2,3
Heidi Schwarzenbach,4 and Klaus Pantel4


Figure 3

Editing of the circulating tumor cell (CTC) pool by the microenvironment of secondary metastatic sites. Tumor cells leave the primary tumor and circulate through the bloodstream. Each time the CTCs reach a new niche (distant organs, e.g., bone, liver, or lung in breast cancer), they undergo an organ-specific nivemesis and may leave this site with a new organ-specific signature. Abbreviation: DTC, disseminated tumor cell.
Somatic mutations
Mutations only metastases
Constitutional mutation
CTC specific mutation
Circulating Tumour Cell Plasticity: Model of metastasis initiating cells.

Plasticity of disseminating cancer cells in patients with epithelial malignancies

Natalia Fedorchuk-Kovt· Catherine Alis-Franetores· Kristina Pantel

Single-Cell Analysis of Circulating Tumor Cells Identifies Cumulative Expression Patterns of EMT-Related Genes in Metastatic Prostate Cancer

Chung-Chang Chen,1 Devi Prasad Mahalingam,2 Pawel Obrzutko,3 Robert K. Jadlow,4 Chuan-Min Wang,1 Robin J. Leech,5–7 Tien-Chung Chang,8 Steven D. Weitman,5,9 Addambala Prasad Raman,6 LuZhe Sun,3 Maria E. Gancayco,1 Ian M. Thompson,5 and Tim Hau-Kit Huang4

Department of Molecular Medicine, Cancer Therapy and Research Center, University of Texas Health Science Center, San Antonio, Texas

The Prostate 73:813–826 (2013)

Fig. 3. Heterogeneous expression profiles of EMT-related and other genes among CTCs. RNA from CTCs was subjected to microfluidics-based single-cell qRT-PCR analysis using a Biomark HD system. Gene expression for each gene was obtained as described in Materials and Methods Section and displayed in a blue-white gradient. Gene symbols and gene groups were labeled on the top and CTC numbers and patient groups on the right. EMT-related genes are further divided into two groups: the frequently expressed group and the less frequently expressed group.
Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition

Min Yu,1,6+ Aditya Bardia,3,5+ Ben S. Wittner,1 Shannon L. Stett,1,2 Malgorzata E. Smes,3
David F. Tsang,2 Steven J. Isakoff,3,5 Jordan C. Ciciliano,1 Marissa N. Wells,3 Ajay M. Shah,2
Kyle F. Concannon,5 Maria C. Donaldson,5 Leida V. Sequist,3,5 Elena Brachtel,1,4
Dennis Sigel,1,4 Jose Baserga,5 Sridhar Ramaswamy,1,3 Mehmet Toner,2,5
Daniel A. Haber,3,5+ Shyamala Maheswaran1,3,5

Epithelial-mesenchymal transition (EMT) of adherent epithelial cells to a migratory mesenchymal state has been implicated in tumor metastasis in preclinical models. To investigate its role in human cancer, we characterized EMT in circulating tumor cells (CTCs) from breast cancer patients. Rare primary tumor cells simultaneously expressed mesenchymal and epithelial markers, but mesenchymal cells were highly enriched in CTCs. Serial CTC monitoring in 11 patients suggested an association of mesenchymal CTCs with disease progression. In an index patient, reversible shifts between these cell fates accompanied each cycle of response to therapy and disease progression. Mesenchymal CTCs occurred as both single cells and multicellular clusters, expressing known EMT regulators, including transforming growth factor (TGF)-β pathway components and the FOXO transcription factor. These data support a role for EMT in the blood-borne dissemination of human breast cancer.

www.sciencemag.org  SCIENCE VOL 339 1 FEBRUARY 2013
Phenotype of CTCs change dynamically with treatment response and treatment resistance

Epithelial

Mesenchymal
CTCs: Clinical promise and insights into dynamics cancer to help shape the pathway to precision oncology

- CTCs offer an minimally invasion approach for prognostication, and treatment selection
- Patients and Tumour cells are heterogeneous
- Cancer progression is highly dynamic due to plasticity and selection pressures
- Cellular and molecular analyses of CTCs can inform treatment options and oncology management with greater precision

Adapted from S Stott, Mass Gen Hosp, Harvard