Circulating Tumor Cells (CTC) in Cancer: a Great Leap Forward

Essay

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DEDICATION

This work is whole-heartedly dedicated to my wife ***Rania****, the light guiding my rood, and my daughter* ***Eleanor****, the hope and joy, for their patience and endurability.*

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List of Abbreviations

ALDH	Aldehyde Dehydrogenases
AMACR	Alpha-Methylacyl-CoA Racemase
AR	Androgen Receptor
AUC	Area Under The Curves
BCSS	Breast Cancer Specific Survival
BL	Baseline
BM	Bone Marrow
BEAMing	Beads, Emulsion, Amplification, and Magnetics assay
CDER	Center For Drug Evaluation And Research
CEA	Carcinoembryonic Antigen
CEC	Circulating Endothelial Cells
CI	Confidence Interval
CK	Cytokeratin
CMP	Committed Myeloid Progenitors
c-MET	Is a Hepatocyte Growth Factor Receptor
CNHC	Circulating Non-Haematological Cells
CRPC	Castrate Resistant Prostate Cancer
CSCs	Cancer Stem Cells
CTCs	Circulating Tumor Cells
ctDNA	Circulating Tumor Deoxyribonucleic Acid
ctRNA	Circulating Tumor Ribonucleic Acid
CTSC	Circulating Tumour Stem Cell
DAPI	4,2-Diamidino-2- Phenylindole Dihydrochloride
DCs	Dendritic Cell
DEP	Dielectrophoresis
DDFS	Distant Disease-Free Survival
DFS	Disease-Free Survival

DTCs	Disseminated Tumor Cells
ECM	Extracellular Matrix
EFS	Event-Free Survival
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial To Mesenchymal Transition
EpCAM	Epithelial Cell Adhesion Molecule
EPhB4	Ephrin Type-B 4 Gene
ER	Estrogen Receptor
ETaR	Endothelin-A Receptor
FACS	Fluorescence-Activated Cell Sorting
FAK	Focal Adhesion Kinase
FDA	Food And Drug Administration
FGF	Fibroblast Growth Factor
FISH	Fluorescence In Situ Hybridization
GalNAc-T	N-acetylgalactosaminyltransferase
GFs	Growth Factors
GSK-3β	Glycogen Synthase Kinase 3 Beta
HER2/neu	Human Epidermal Growth Factor Receptor 2
H/E(Spl)	Hairy/ Enhancer Of Split
HGF	Hepatocyte Growth Factor
HIFs	Hypoxia-Inducible Factors
HPC	Hematopoietic progenitor cell
HR	Hazard Ratio
IF	Immunofluorescence
IGF	Insulin Like Growth Factor
IL-13	Interleukin number 13
IL-4	Interleukin number 4

ILK	Integrin-Linked Kinase
IPASS	IRESSA Pan-Asia Study Results
LDH	Lactate Dehydrogenase
MAGE-A3	Melanoma-associated antigen 3
MAPK	Mitogen-Activated Protein Kinase;
mCRC	Metastatic Colorectal Cancers
MDR	Multidrug Resistance
MDSCs	Myeloid-Derived Suppressor Cells
MET	Mesenchymal To Epithelial Transition
MFS	Metastasis-Free Survival
MMPs	Matrix Metalloproteinases
MRD	Minimal Residual Disease
mRNAs	Messenger Ribonucleic Acid
MSI	Microsatellite Instability
MSKCC	Memorial Sloan-Kettering Cancer Center
MYC	Myelocytomatosis Oncogene
NF-kB	Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells
NSCLC	Non Small Cell Lung Cancer
NT	Neoadjuvant Treatment
OS	Over All Survivial
PAR6	Partitioning-Defective Protein-6
PC	Prostate Cancer
pCR	Pathological Complete Response
PFS	Progression Free Survival
PI3K	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase
PKB	Protein Kinase-B
PR	Progesterone Receptor

pRb	Retinoblastoma Protein
PSA	Prostate Specific Antigen
PSMA	Prostate Specific Membrane Antigen
qRT-PCR	Quadriplex Real-Time Polymerase Chain Reaction
RAF	Rapidly Accelerated Fibrosarcoma
RAS	Rat Sarcoma
RECIST	Response Evaluation Criteria In Solid Tumours
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SARMS	Scorpion Amplification Refractory Mutation System
SBEM	Small Breast Epithelial Mucin
SCLC	Small Cell Lung Cancer
SEER	Surveillance And Epidemiology End Results
SEM	Scanning Electron Microscopy
TAK1	TGF-β Activated Kinase-1
TAMs	Tumor-Associated Macrophages
TGF-α	Transforming Growth Factor Alpha
TGF-β	Transforming Growth Factor Beta
TGF-βR	TGF-β Receptor
TKI	Tyrosine Kinase Inhibition
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor Alpha
V-CAM	Vascular Cell Adhesion Molecule
VEGF	Vascular Endothelial Growth Factor
Wnt	Int/Wingless Family (Combination Of Wingless-Related Integration Site)
Wnt-R	Wnt Receptor

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INTRODUCTION

Despite all efforts, cancer still remains a principal cause of death worldwide. In 2008, cancer claimed 7.6 million lives (~13% of all human deaths), and this number is expected to pass 13 million in 2030 **(Jemal et al., 2011).** According to the World Health Organization, however, at least 30% of these deaths are preventable **(Esmaeilsabzali et al., 2013).** In majority of cases, it is not the primary tumor but its spread to distant organs that eventually compromises the function of the host organ and is associated with mortality. For instance, the average 5-year survival rate for patients with localized and metastatic prostate cancers is 100% and 28%, respectively **(American Cancer Society, 2013).**

A typical cancerous tumor contains millions or even billions of cells harboring genetic mutations driving them to grow, divide, and invade the local tissue in which they're embedded. However, as the cells proliferate, they don't all stay in the neighborhood. Some cells slough off the edges of a tumor and are swept away by the bloodstream or lymphatic system. These so-called circulating tumor cells (CTCs) can remain loose in circulation, cluster together as they travel, or lodge themselves in new tissues. Whatever their path, their common origin means that CTCs hold information about a tumor, information that researchers think could be key to cancer diagnosis or treatment (**Williams 2013**).

Bone Marrow (BM) appears to be a common homing organ for Disseminated Tumor Cells (DTCs) derived from carcinomas of different organs; it also might be a reservoir for DTCs with the capacity to reenter other distant organs **(Pantel, and Brakenhoff, 2004).** Minimal residual

disease (MRD; i.e., the presence of DTCs) is undetectable by high-resolution imaging technologies; however, DTCs can now be identified in the BM, lymph nodes, or circulating blood with sensitive and specific assays **(Pantel et al., 2009).**

For the follow-up of patients with cancer, sequential analyses are pivotal. Because BM needle aspiration is far more invasive than sampling peripheral blood, research groups are currently evaluating the clinical utility of testing for tumor cells in the blood instead of the BM to assess prognosis and monitor systemic therapy **(Pantel et al., 2009).**

Since they were first described by Thomas Ashworth in 1869, the presence of circulating tumor cells (CTCs) has been suggested to be associated with cancer by various early studies **(Ashworth, 1869; Carey et al., 1976; Myerowitz et al., 1977; Gallivan, and Lokich, 1984).** Although epithelial CTCs were first described well over 100 years ago **(Ashworth, 1869),** only recently has CTC enumeration been shown to be clinically useful as a prognostic biomarker in epithelial malignancies **(Harouaka et al., 2014).**

The detection and molecular characterization of circulating tumor cells (CTCs) are one of the most active areas of translational cancer research, with more than 400 clinical studies having included CTCs as a biomarker. The aims of research on CTCs include (a) estimation of the risk for metastatic relapse or metastatic progression (prognostic information), (b) stratification and real-time monitoring of therapies, (c) identification of therapeutic targets and resistance mechanisms, and (d) understanding

metastasis development in cancer patients **(Alix-Panabières, and Pantel, 2013).**

Several systems have been developed for isolation and characterization of CTCs from blood samples including the FDA approved CellSearch platform **(Hughes, and King, 2012; Yu et al., 2011).** Using these platforms, CTCs have been isolated from blood samples of patients with a variety of tumors including head and neck **(Nichols et al., 2011),** breast **(Swaby, and Cristofanilli, 2011),** lung **(O'Flaherty et al., 2011),** colorectal, gastric, pancreatic **(Takeuchi, and Kitagawa, 2010),** renal cell, urinary bladder, and prostate cancers **(Kruck et al., 2011).**

Many studies have showed significant correlation between higher CTC counts and shorter overall survival (CTC counts above a known threshold are a prognostic marker and predictor of patient outcome) in patients with metastatic breast **(Cristofanilli et al., 2004; Hayes et al., 2006),** lung **(Miller et al., 2010)**, prostate **(Danila et al., 2007; De Bono et al., 2008),** and colon cancer **(Cohen et al., 2008).**

Based on these clinical trials, the US Food and Drug Administration (FDA) cleared the CellSearch technology (Veridex, LLC, Raritan, NJ, USA) for CTC enrichment and enumeration for the above four indicated cancers. The success of CellSearch proves that enumeration of CTCs is indeed a surrogate for active disease and that increased CTC numbers are predictive of worse prognosis. In comparison to traditional metastatic tissue biopsy, isolation of CTCs offers numerous advantages: collection of peripheral blood is easy to perform, relatively inexpensive, presents significantly less

risk to patients, is less morbid, and is easily repeatable over time **(Harouaka et al., 2014).**