Identifying genomic signatures in circulating breast tumour cells

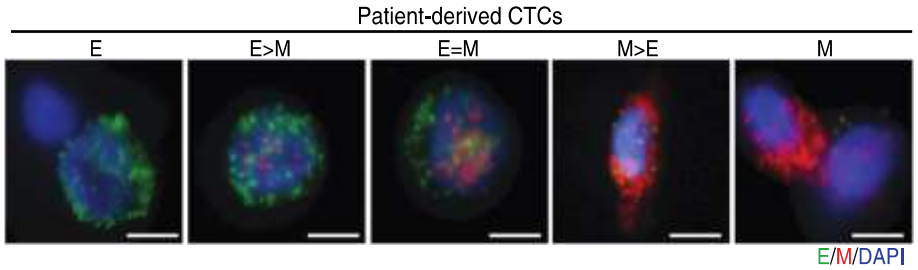
9th ISMRC 2013, Paris, France
September 25th, 2013

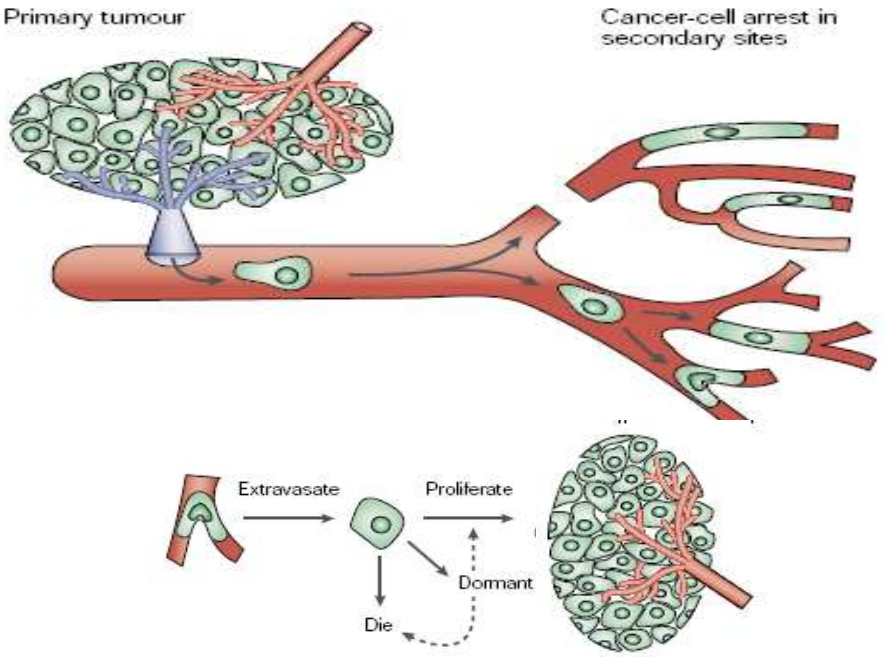
NISHA KANWAR
PhD Candidate

Department of Laboratory Medicine and Pathobiology
University of Toronto
The Campbell Family Institute for Breast Cancer Research at the Princess Margaret Cancer Centre, Toronto, Canada

Defining CTCs, currently..

- Molecular imaging of enumerate and monitor CTC levels in blood
- Proven prognostic value in early and metastatic breast cancer (n=6825; *Zhang L., Cli Cancer Res, 2012*)
- Varying degrees of concordance with molecular markers of primary tumours
  - Subclonal detection
- Predictably, individual cells reflect the heterogeneity and evolution of tumour cells during cancer progression
- *Hypothesis*: A degree of commonality is also palusible, highlighting alterations that allow tumour cells to preferentially perform CTC-defining activities
  - Motility
  - Intravasation/extravasation
  - Survival/Chemo-resistance
  - Metastasis



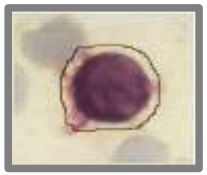


Yu M, Science, 2013; Chambers A F, Nat Rev Cancer, 2002

Genomic characterization of CTCs

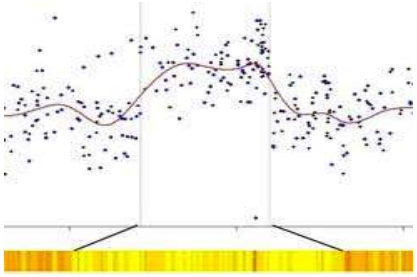
Immunomagnatic sorting of blood
CD45-depleted fraction

Pan-cytokeratin staining

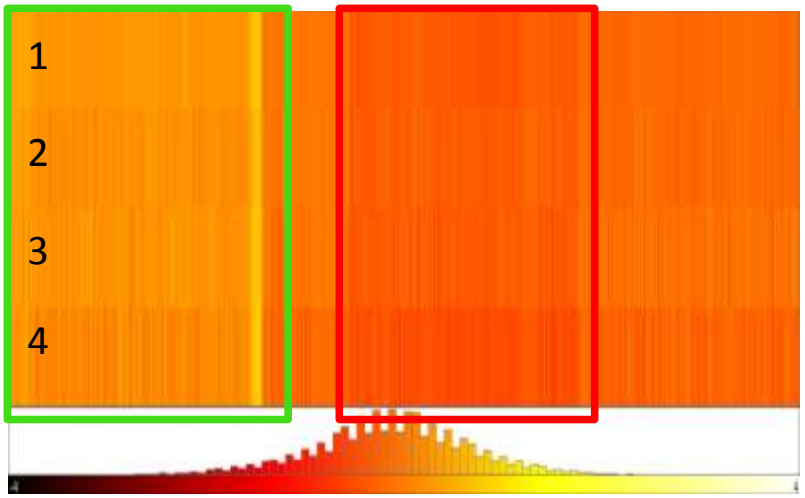


Single-cell laser assisted microdissection

Whole **G**enome **A**mplification



Copy number analysis *Affymetrix SNP 6.0*

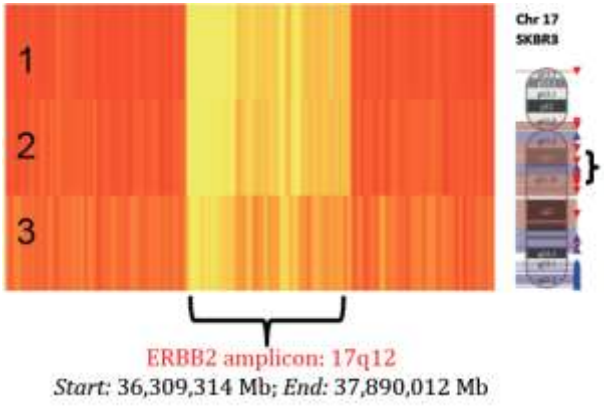


MCF7 breast cancer cells

1 = Unamplified Genomic DNA
2 = WGA DNA from 2 cells
3 = WGA DNA from 3 cells
4 = WGA DNA from 5 cells

Concordance:

genomic DNA / single cells: **86-92%**
single cell / cell: **87-93%**

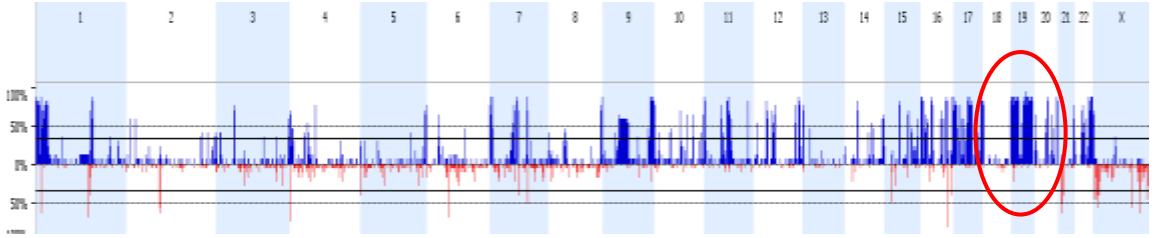


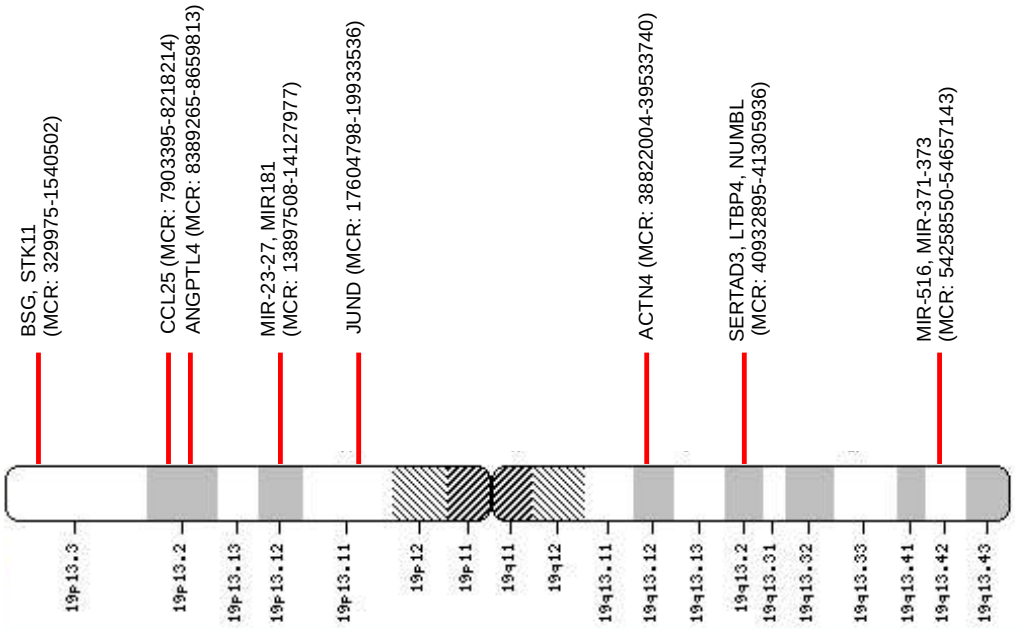
SKRB3 breast cancer cells

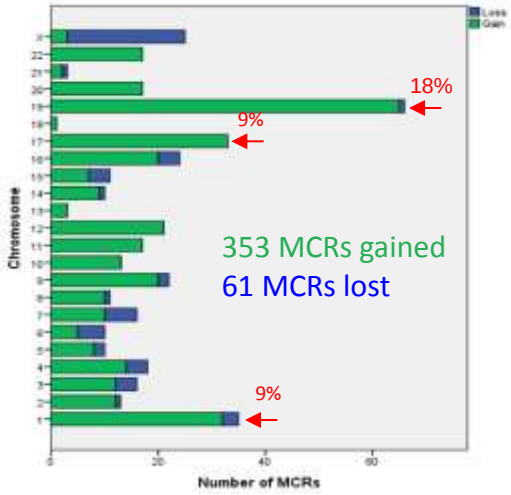
1= Unamplified Genomic DNA
2= WGA genomic DNA
3= WGA DNA from 2 cells

Signature of Recurrent gains in CTCs

n = 17 CTC / normal pairs





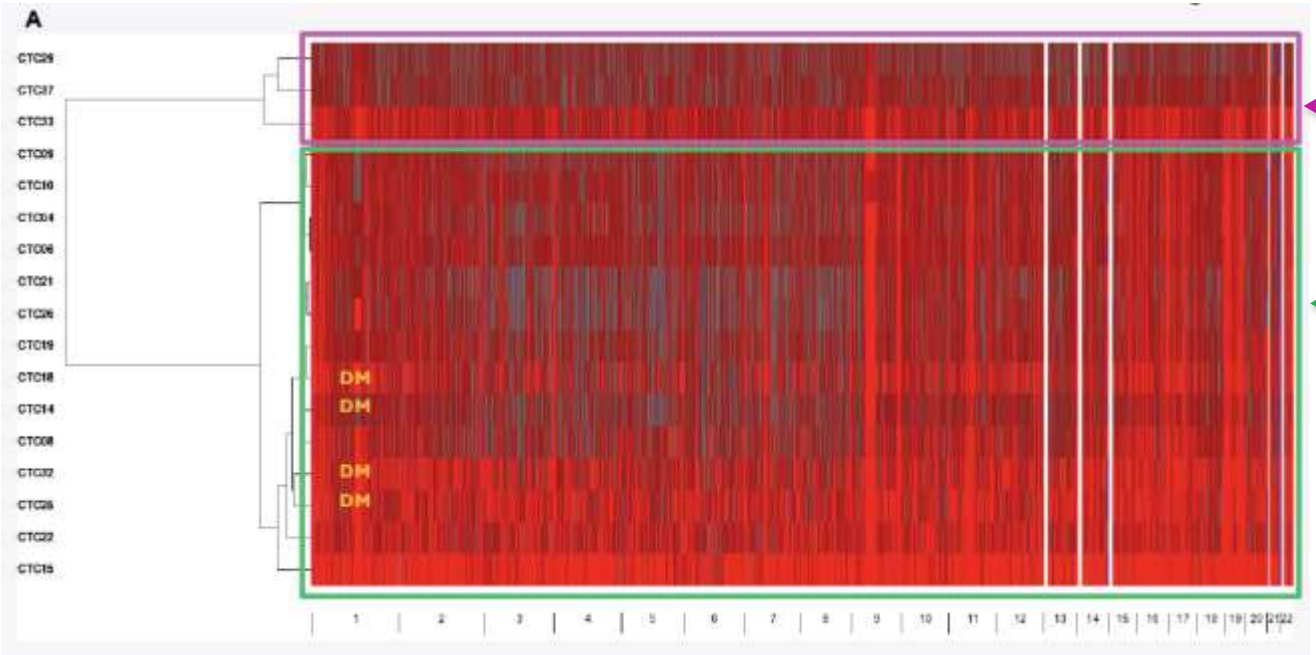


Minimal Common Regions of gain in at least 4 samples

- **90 MCRs in recurrent signature (15-16/17 samples)**
- 48/90 MCRs on chr 19
- 1 MCR gained in 16/17 samples: ***SERTAD3, LTBP4, NUMBL*** (oncogenic transformation, TGFB activation, metastasis)

1q21-23	*ADAM15,MUC1*
7q22.1	*SERPINE/PAI-1*
11p15.5	*CD151*
17q21	*ERBB2*
17q25.2	*ITGB4*
1p36.3	*PRKCZ*
7p22	*FASCN1*

- Genes with CTC-like functions: invasion, intravasation, survival, resistance to anoikis, chemo-resistance
- Examined 787 primary invasive breast carcinomas: CTC-like MCRs present in **3-4%** samples: **CCNE1, KLK7-12, MIR-500s, MIR-371-373, AKT2**



- Net pro- and anti- tumourigenic gene expression results in a balanced state or dormant CTCs, quiescent, chemo-resistant?
- Aggressive tumour cells propagating dissemination and metastatic disease via CTCs
- Early acquired alterations necessary for survival of CTCs

CLUSTER 1: **Dormancy-related signature**
N=3, 11 MCRs

MIR-602
PTEN
SMAD2
AKT2
CADM2
EPHA5
ESR2

CLUSTER 2: **Tumour-aggressiveness related signature**
N=14, 398 MCRs, 110 found in all samples, 54 on chr19
Distant Metastases (DM) p = 0.07
Age <50 p = 0.06

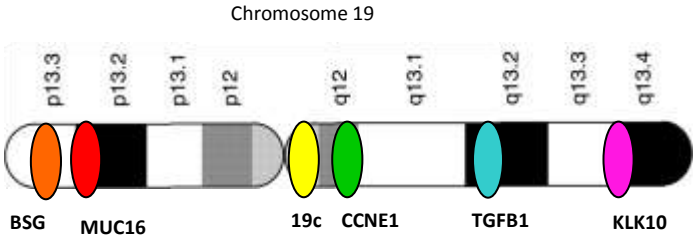
BSG
TUBB4
INSR
CCL25
ANGPTL4
ANGPTL6
JUND
MAG
ACTN4
SIRT2
MARK4
MIR-7, 24/23/27, 181, C19MC and MIR371-73 cluster

Common signature
2 MCRs
Chromosome 21q21 (2-12/17 samples):
ABCG1
COL18A1
COL6A1
CSTB
ITGB2
PRMT2
TFF3

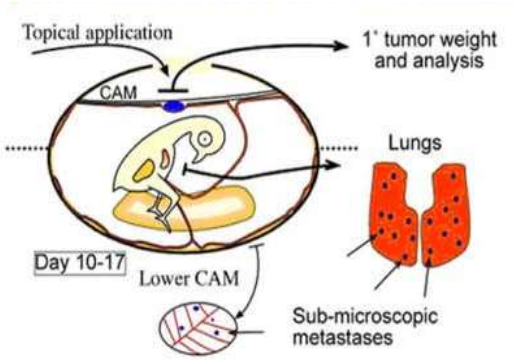
Chromosome 19q13.13 (16/17 samples):
SERTAD3
LTBP4
NUMBL

Validation of CTC-signatures

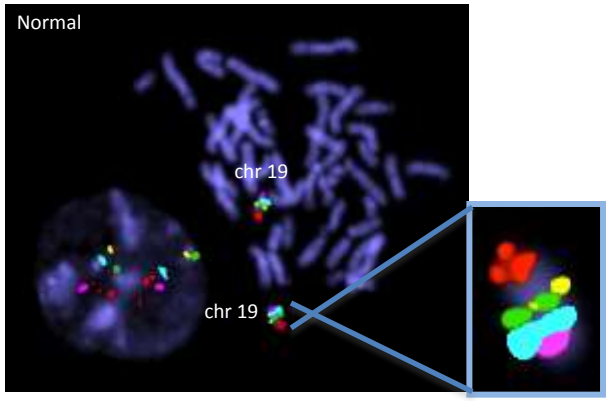
A

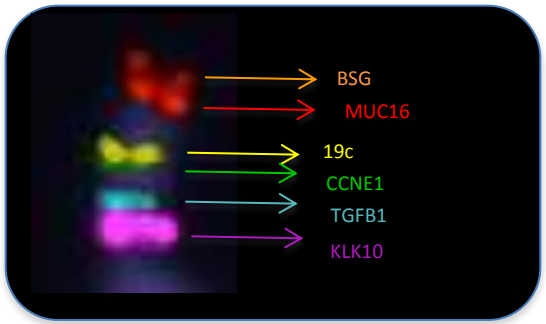


B



Intratumoural assessment: proportion of cells with CTC-like gains





	Chromosomal region	Number of genes in region
Hyb 1	19p13.3	254
	19p13.3-13.2	15
	19p13.2-13.11	379
	19q13.32-13.41	299
	19q13.41	11
Hyb 2	9q34.3	119
	11p15.5-15.4	138
	17q25	108
	17q25	63
	1p36.3	34

A: Multispectral-FISH for recurrent gains signature (10 regions)

B: In vivo chick embryo assay for intravasation and metastasis – 500 genes from recurrent, dormancy and tumour aggressiveness signatures

Acknowledgements

Supervisor: Dr. Susan Done

Funding:

CIHR IRSC
Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

CANADIAN BREAST CANCER FOUNDATION
FONDATION CANADIENNE DU CANCER DU SEIN
Ontario

Lab members:

- Dr. Adeoye Adewunmi
- Ranju Nair
- Moustafa Abdalla
- Dr. Yanglong Zhu
- Dr. Carolina Lopez-Uran

Princess Margaret Cancer Centre, Toronto, Canada:

Dr. Mark Clemons
Dr. Phillipe Bedard
Dr. Eitan Amir
Manoj Mathews

Bioinformatics:

- Dr. Pingzhao Hu *(TCAG, Toronto) Edmonton*

Chick embryo asays:

Dr. John Lewis *(University of Alberta, Canada)*