Circulating Biomarkers - CTCs

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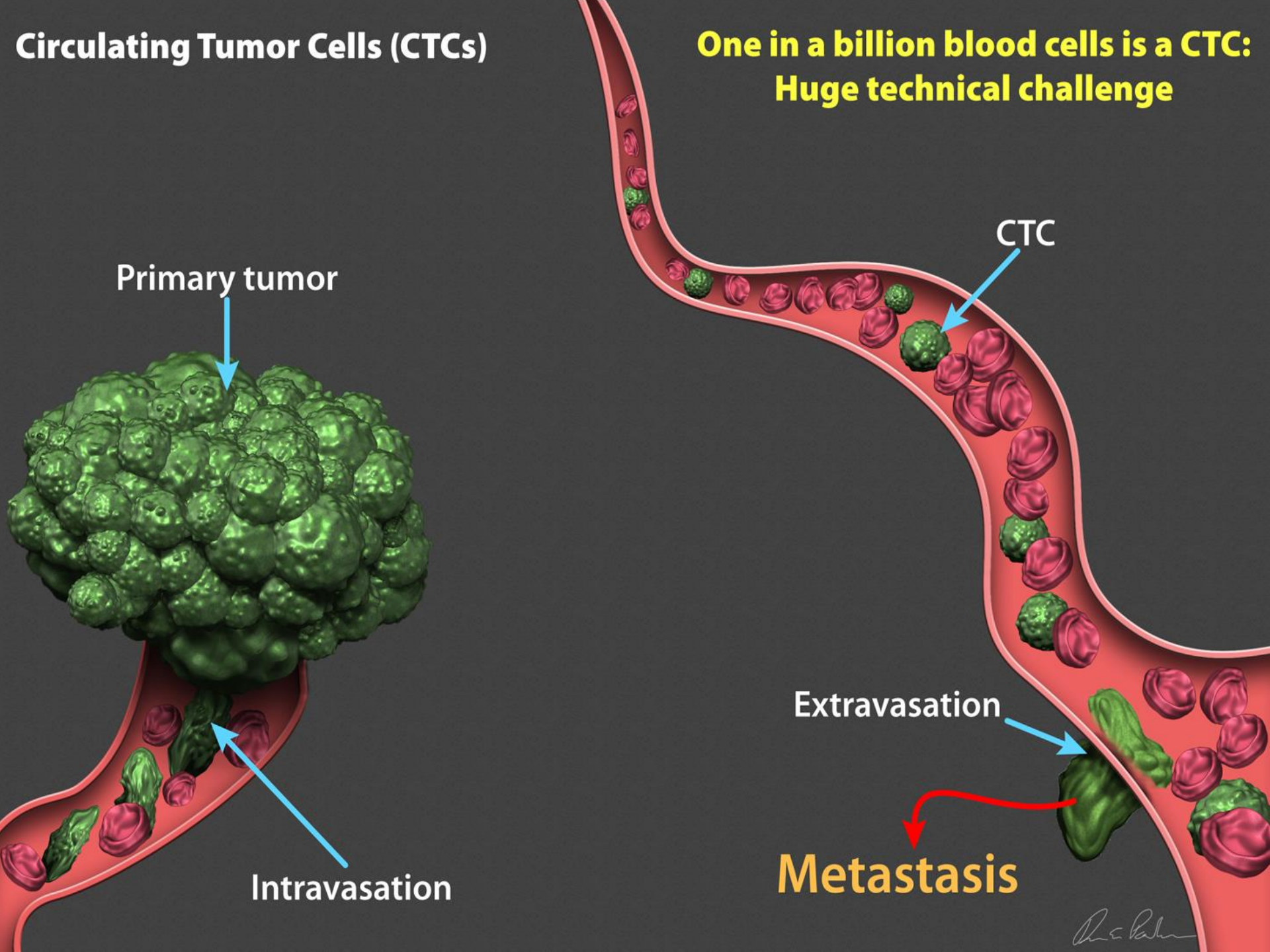
DISCLOSURE SLIDE

- Inventor on patent WO2015061091A1 “Methods relating to Circulating Tumor Cell Clusters and the Treatment of Cancer” filed by The General Hospital Corporation, Boston, USA

- Inventor on patent WO2013144240A1 “Inhibition of Interleukin-8 and/or its Receptor CXCRL in the Treatment of HER2/HER3-overexpressing Breast Cancer” filed by The Friedrich Miescher Institute, Switzerland

- Inventor on patent WO2012168259A1 “Protein Tyrosine Phosphatase, non-receptor Type 11 (PTPN11) and Triple Negative Breast Cancer” filed by The Novartis Research Foundation, Switzerland

- Inventor on patent WO2011120902A1 “Protein Tyrosine Phosphatase, non-receptor Type 11 (PTPN11) and Tumor Initiating Cells” filed by The Novartis Research Foundation, Switzerland


Circulating Tumor Cells (CTCs)
One in a billion blood cells is a CTC:
Huge technical challenge
CTC
Primary tumor
Extravasation
Metastasis
Intravasation

Presentation Outline

1. **Technologies** for CTC capture

2. Investigating **CTC-clusters**

3. Ex vivo **culture of CTC** for individualized testing of drug susceptibility

4. **What's next**

10 mL Whole Blood

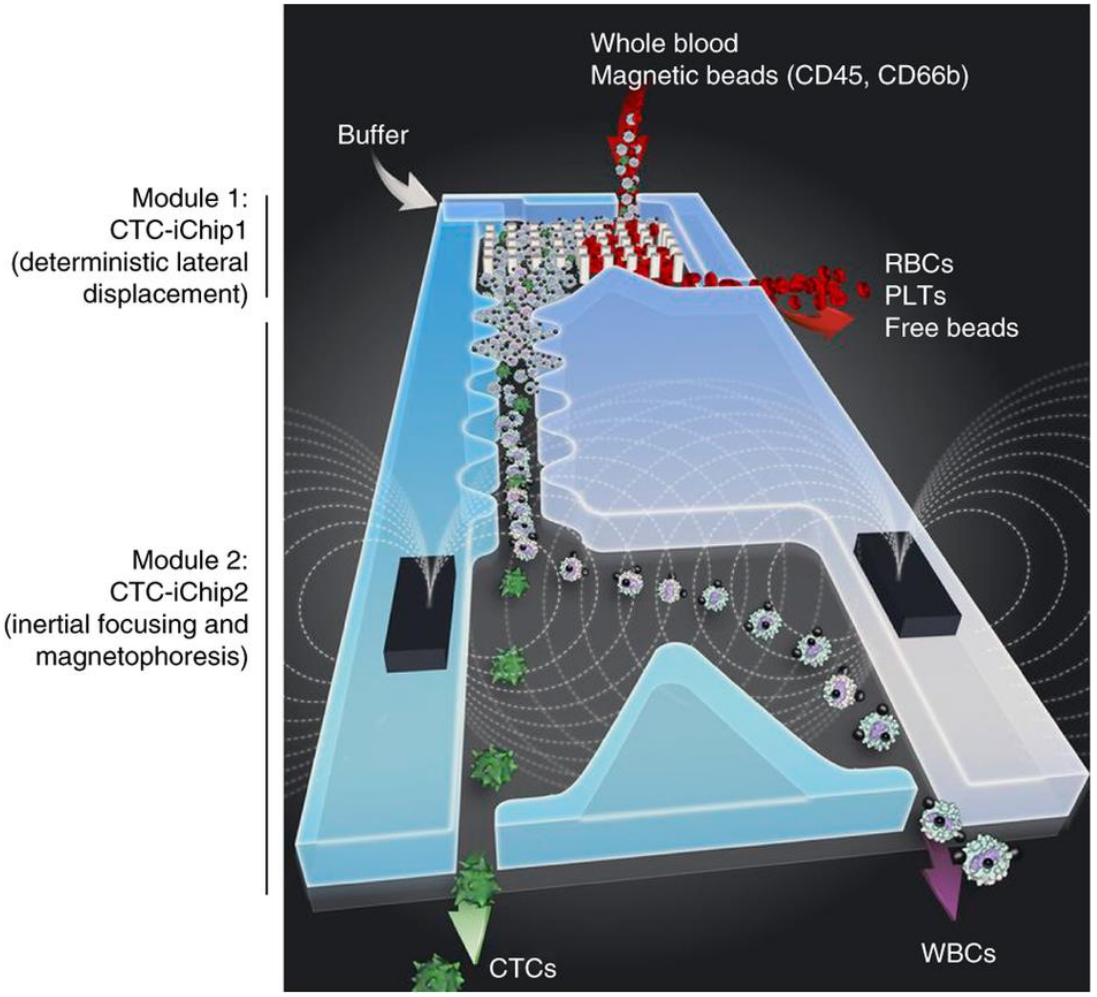
50 billion RBCs

50 million WBCs

0-100 CTCs

1. Microfluidic Capture of CTCs

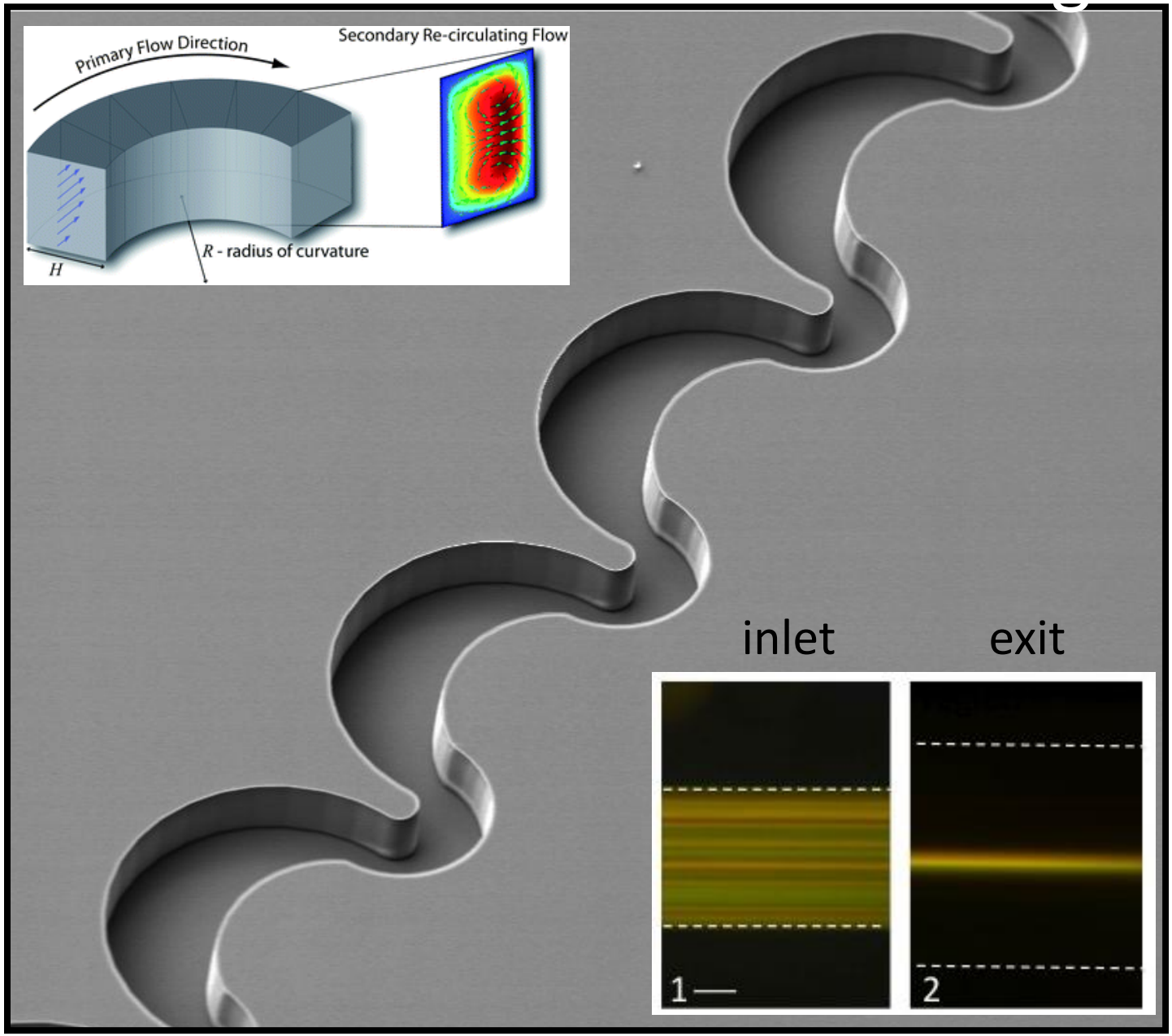
(iChip - collaboration with Dr. Mehmet Toner, Harvard)



Ozkumur et al., ***Science Transl Med***, *2013*

Karabacak et al., ***Nature Prot***, *2014*

Inertial Focusing of Cells in Microfluidic Channel



Mehmet Toner

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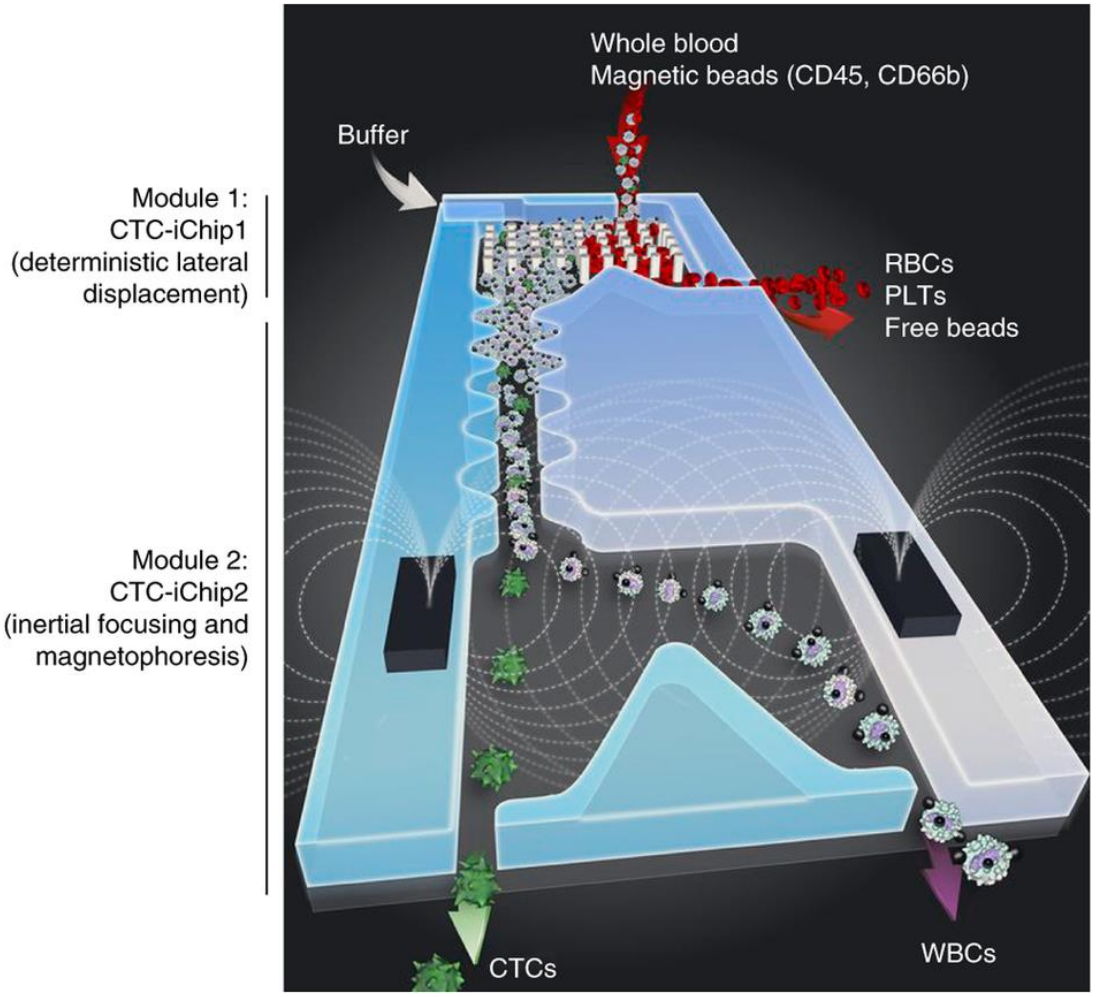

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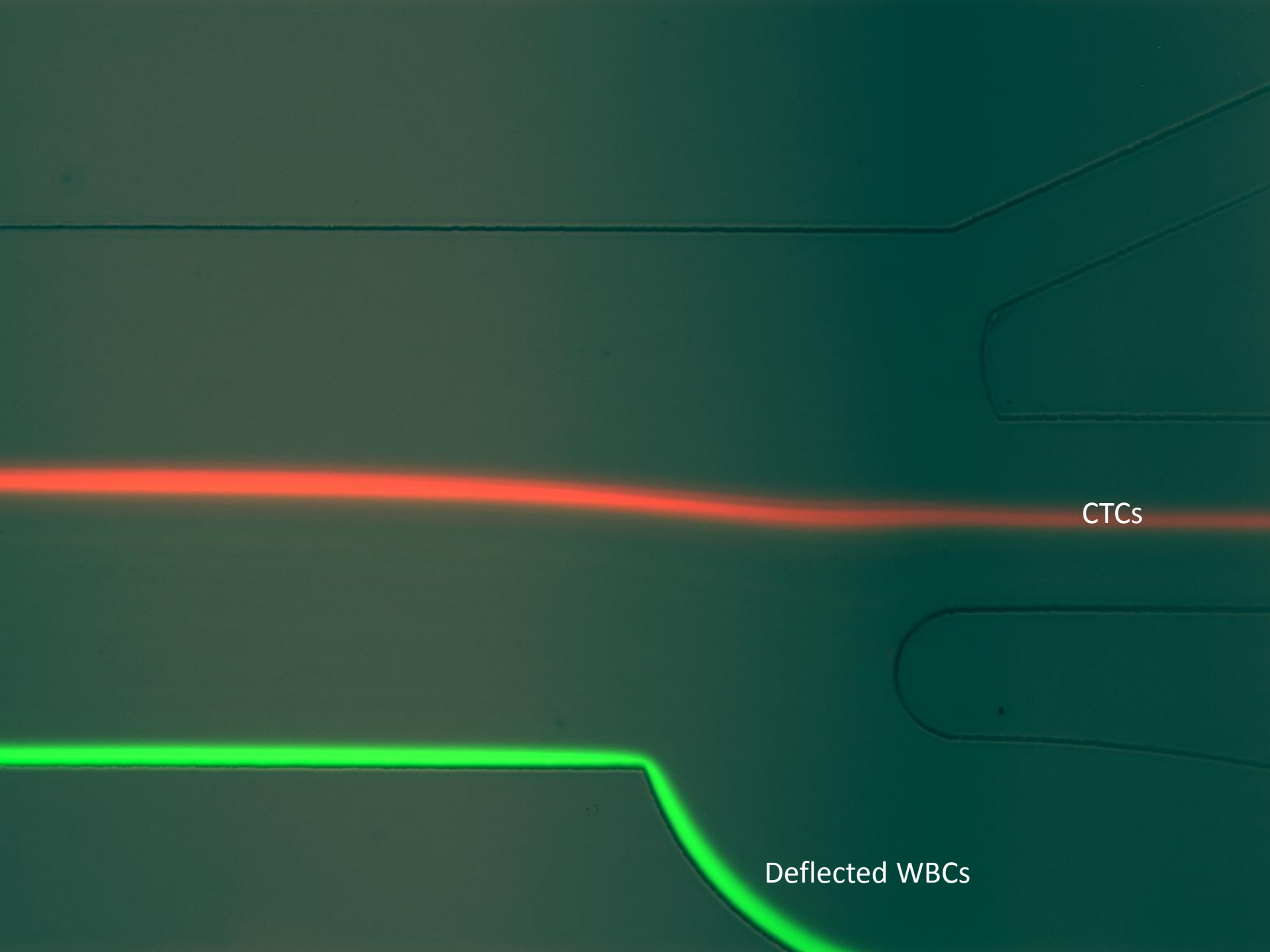
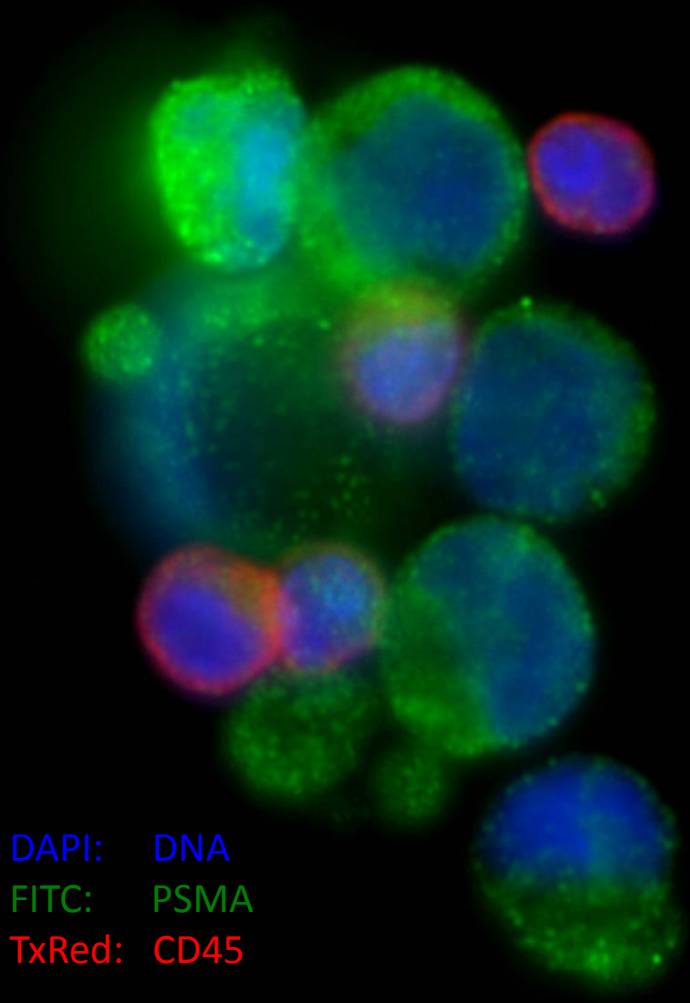

CTCs
Deflected WBCs

2. Clusters of CTCs in the bloodstream



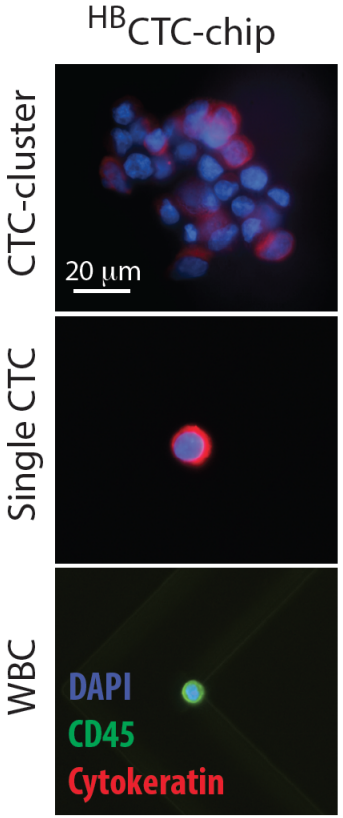
DAPI: DNA
FITC: PSMA
TxRed: CD45

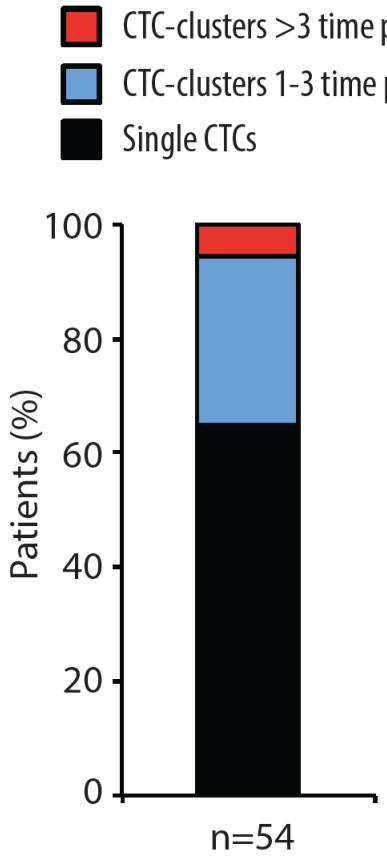
Stott et al., Science Transl Med 2010

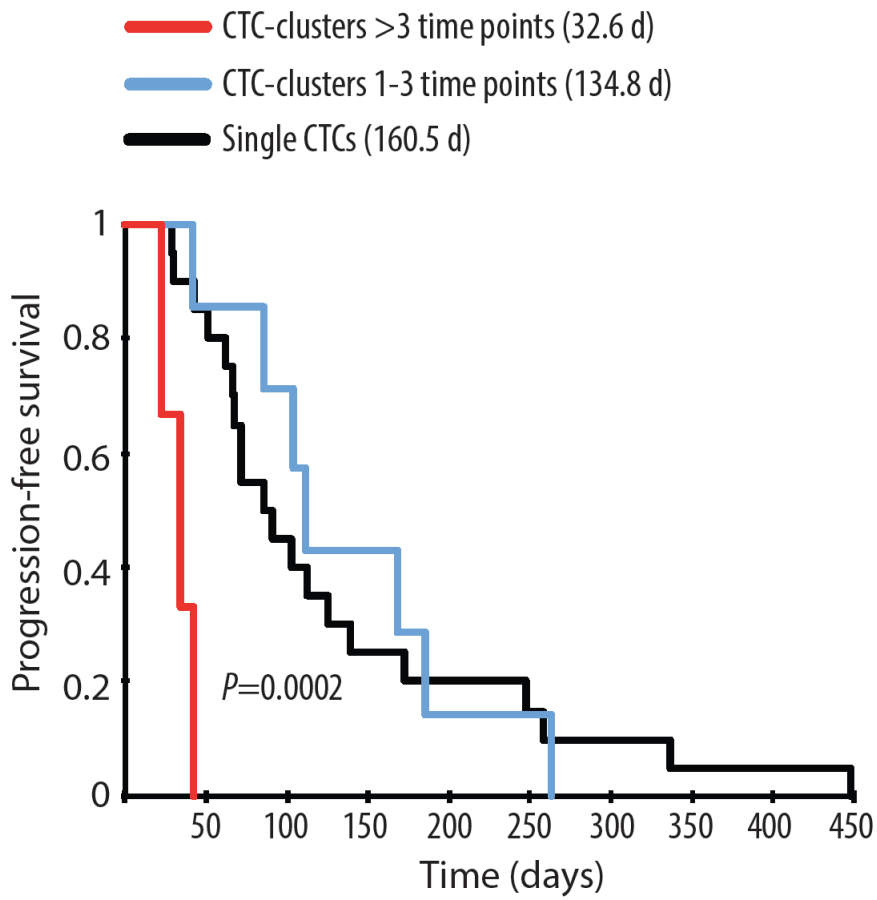
2. Clusters of CTCs in the bloodstream

Are **CTC-Clusters relevant** for the metastatic process?

CTC-Clusters in patients with metastatic breast cancer

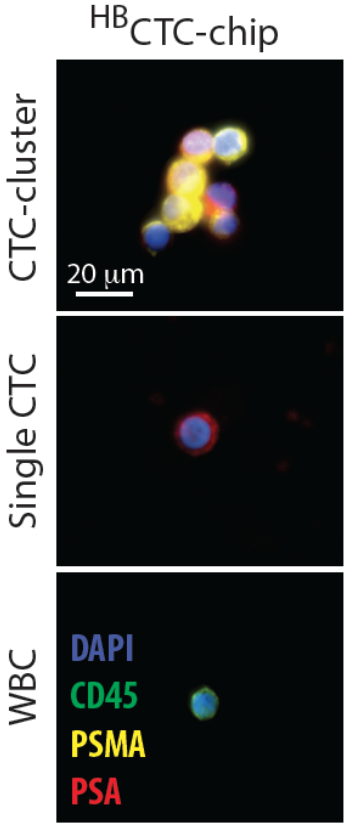


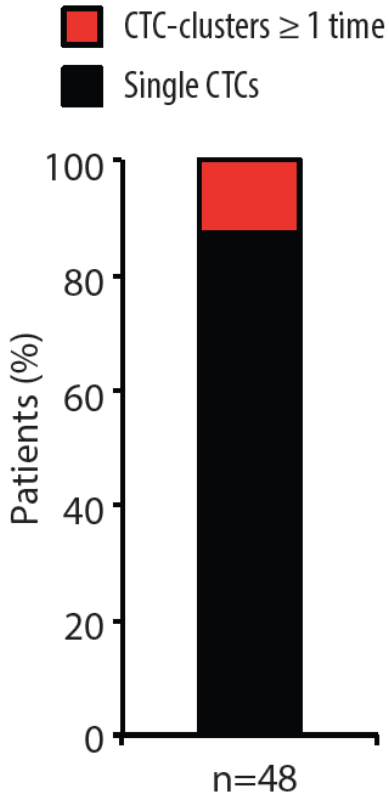


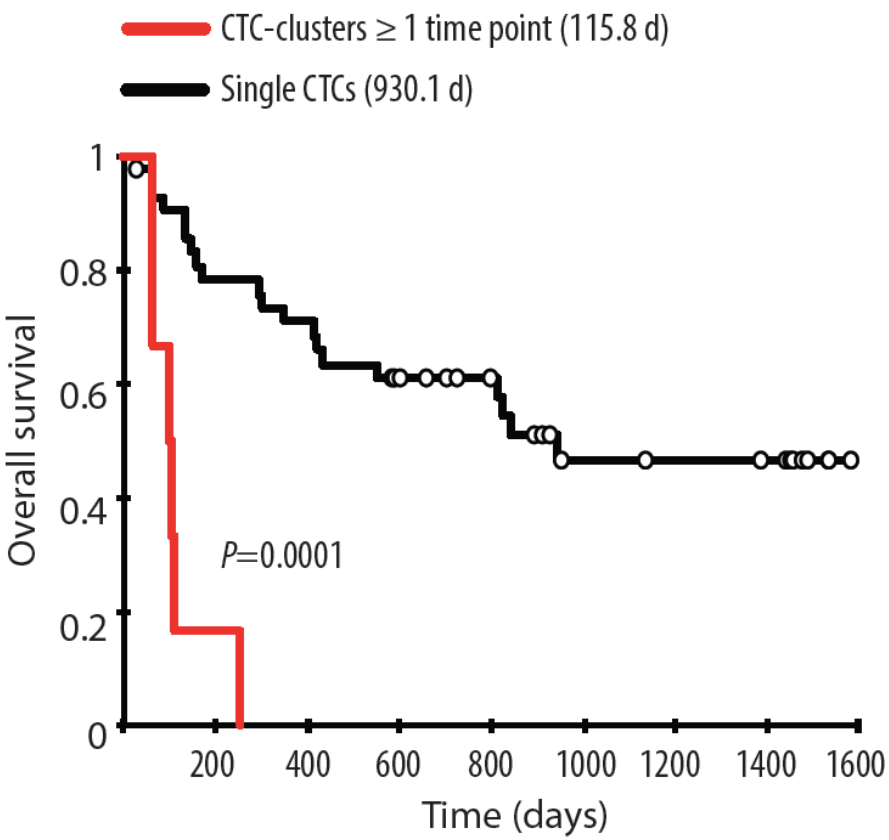


Aceto et al., ***Cell****, 2014*

CTC-Clusters in patients with metastatic prostate cancer







Aceto et al., ***Cell****, 2014*

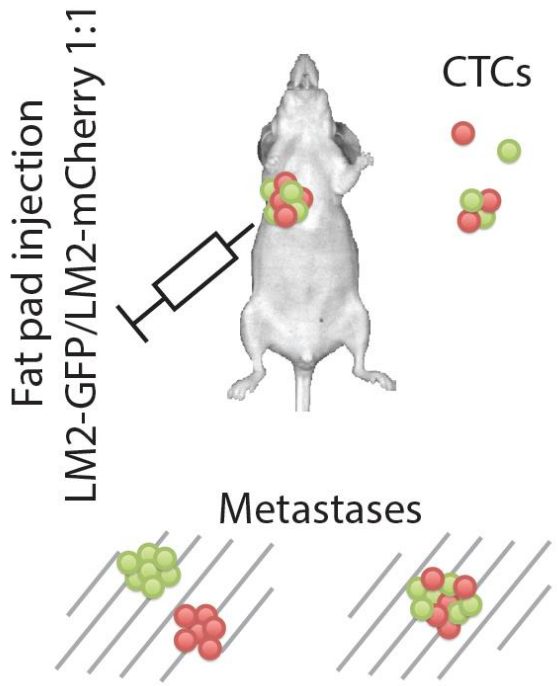
Mouse models

Where do CTC-clusters come from?

What is the metastatic potential of CTC-clusters, compared to single CTCs?

Are CTC-Clusters clonal or oligoclonal?

Mixed primary tumor MDA-LM2 (mCherry/GFP)



*Aceto et al., **Cell**, 2014*

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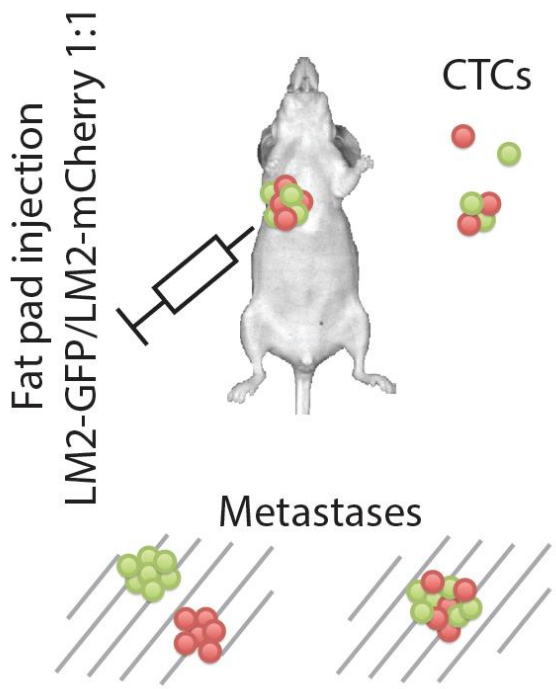
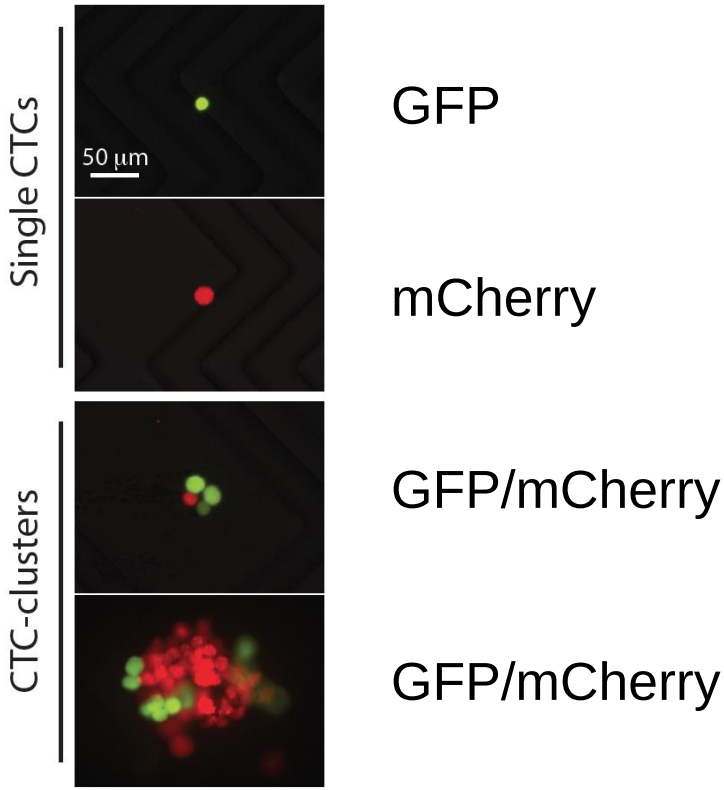


CTC-clusters are mixed origin: mCherry + GFP



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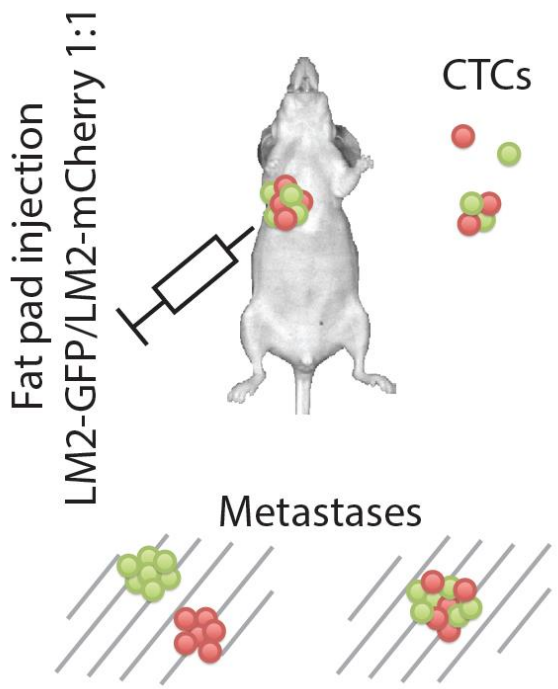
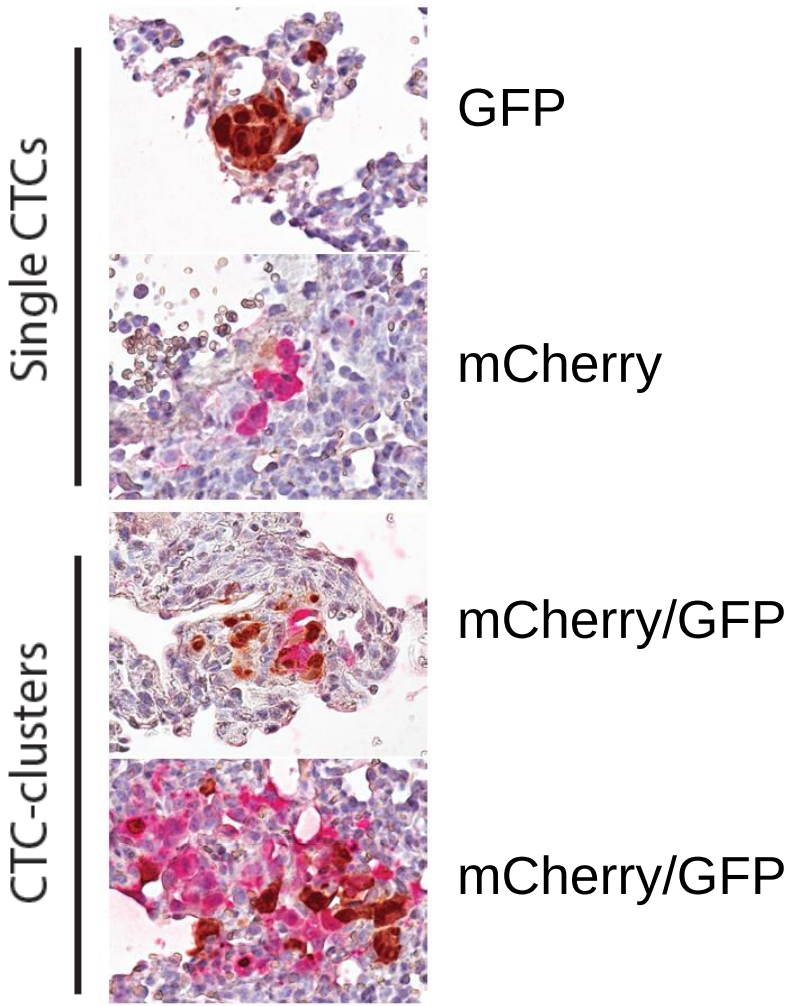


*Aceto et al., **Cell**, 2014*

Metastatic Lung foci



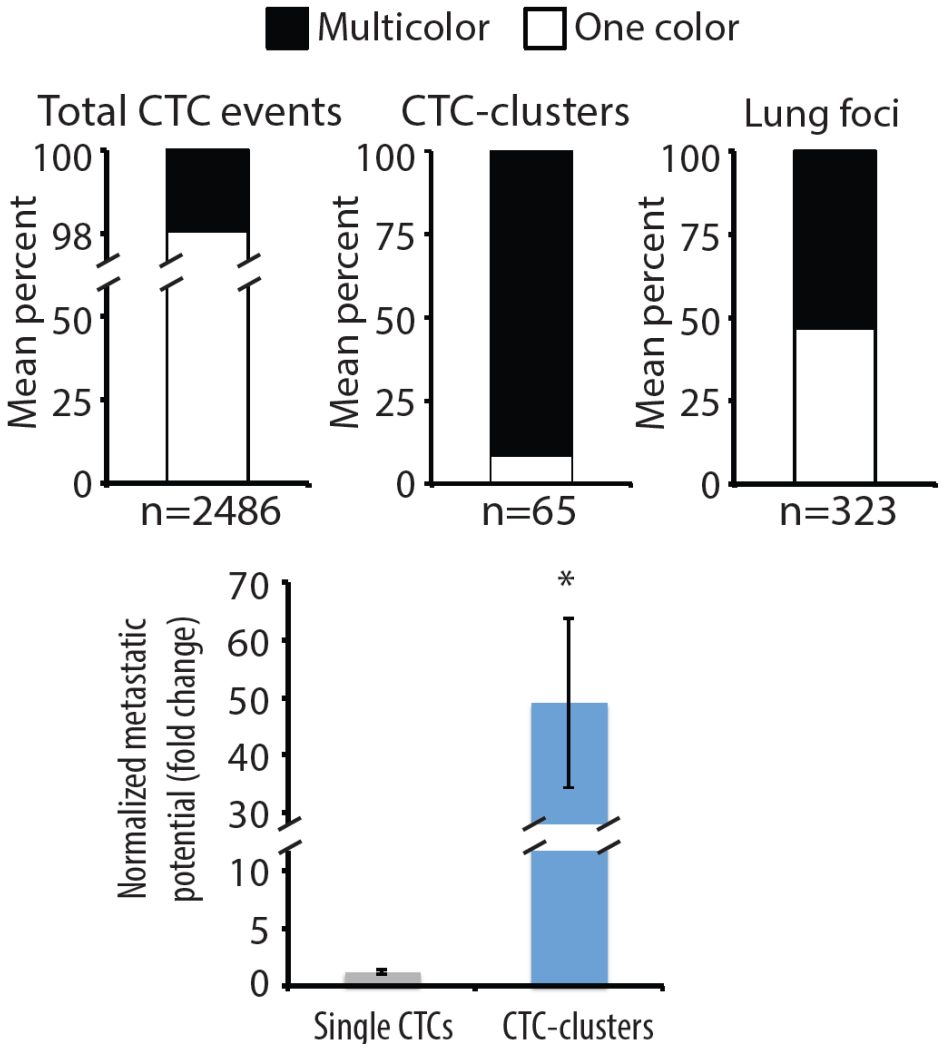
Relative contribution of single CTCs and CTC-clusters to metastatic lesions

2.6% of all CTC events are CTC-clusters

91% of CTC-clusters are multicolored

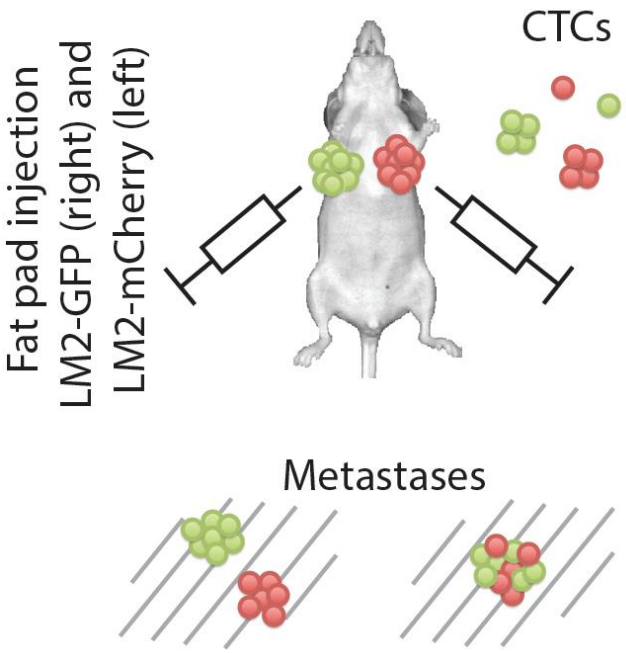
53% of all lung foci are multicolored

CTC-clusters are ~50-fold more metastasis-competent than single CTCs



Do CTC-clusters originate from a single tumor or from intravascular aggregation?

Two single color primary tumors (MDA-LM2)



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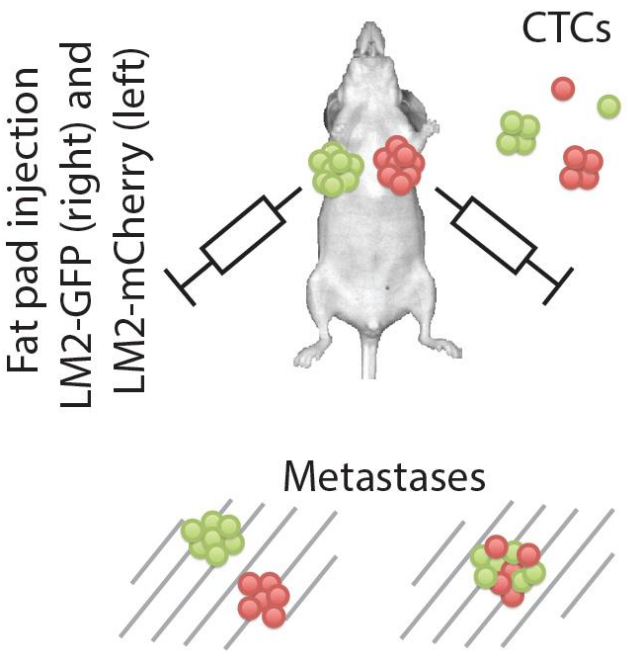
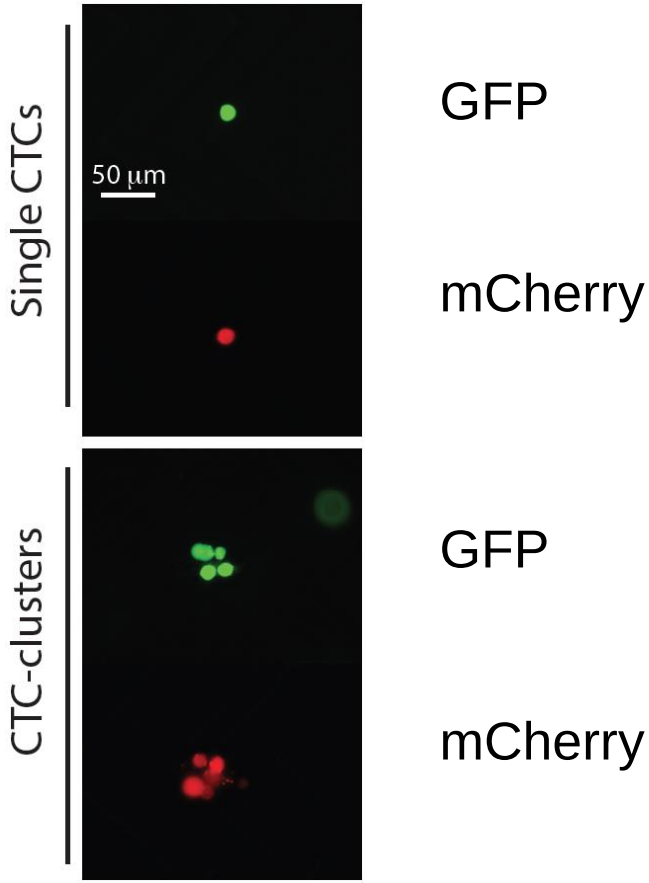


CTC-clusters are derived from only one tumor



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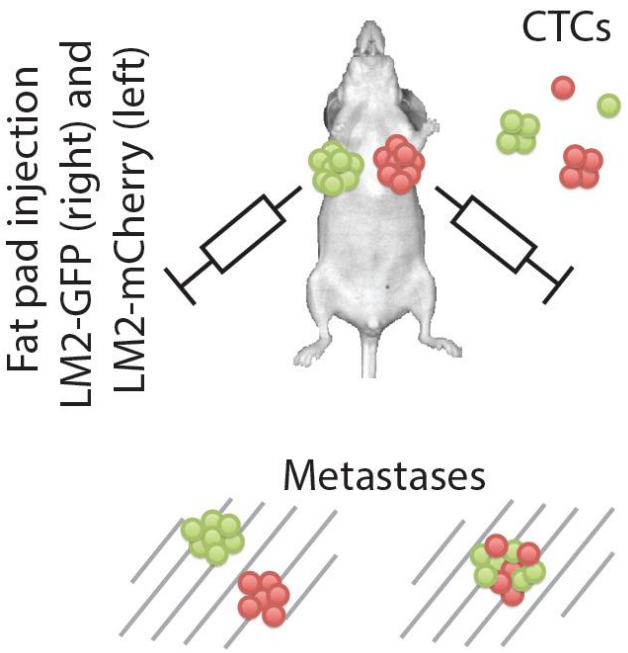
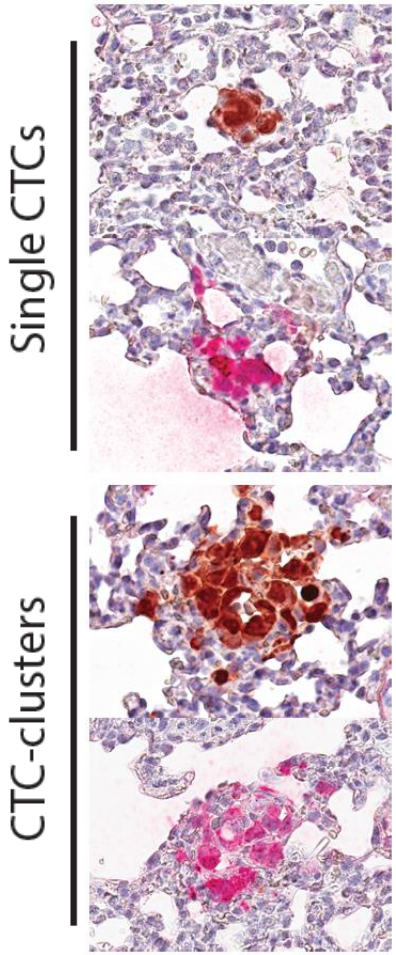
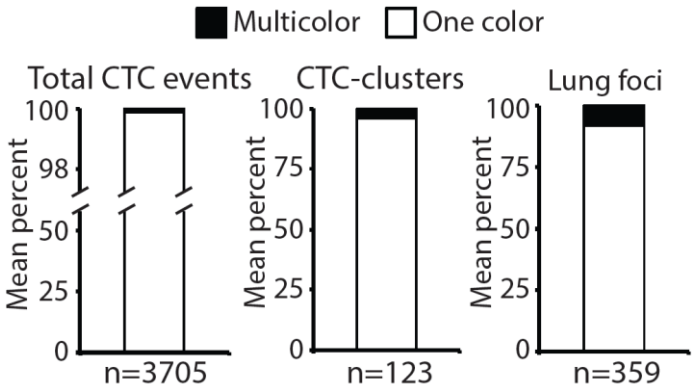


*Aceto et al., **Cell**, 2014*

Lung metastases are primarily derived from one tumor (92%)





Cross-seeding of primary lesions: **3-5%** *(Massague/Norton)*

Re-seeding of metastatic lesions: **8%**

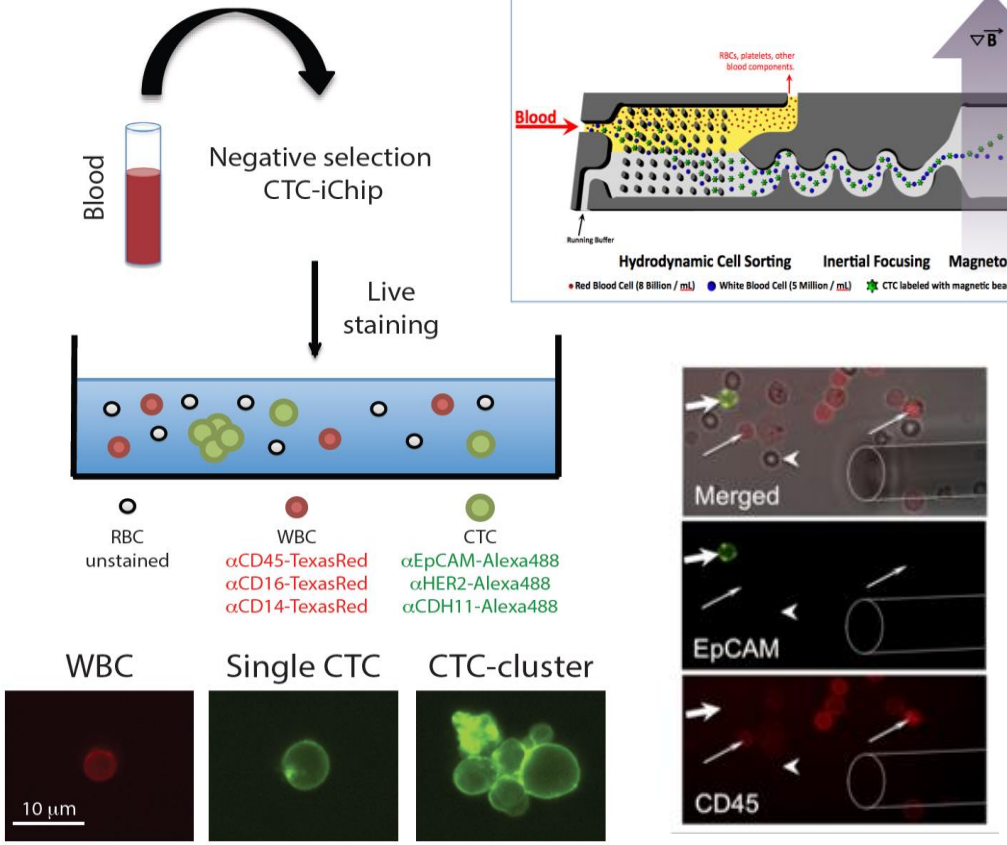
RNA profiling of single CTCs

RNA sequencing of single CTCs *vs* CTC-Clusters from human breast cancer patients

Single cell RNA sequencing of CTCs vs CTC-clusters

CTCs from patients with breast cancers:

- Cell surface staining (EpCAM, HER2, CDH11) for micromanipulator and RNA seq



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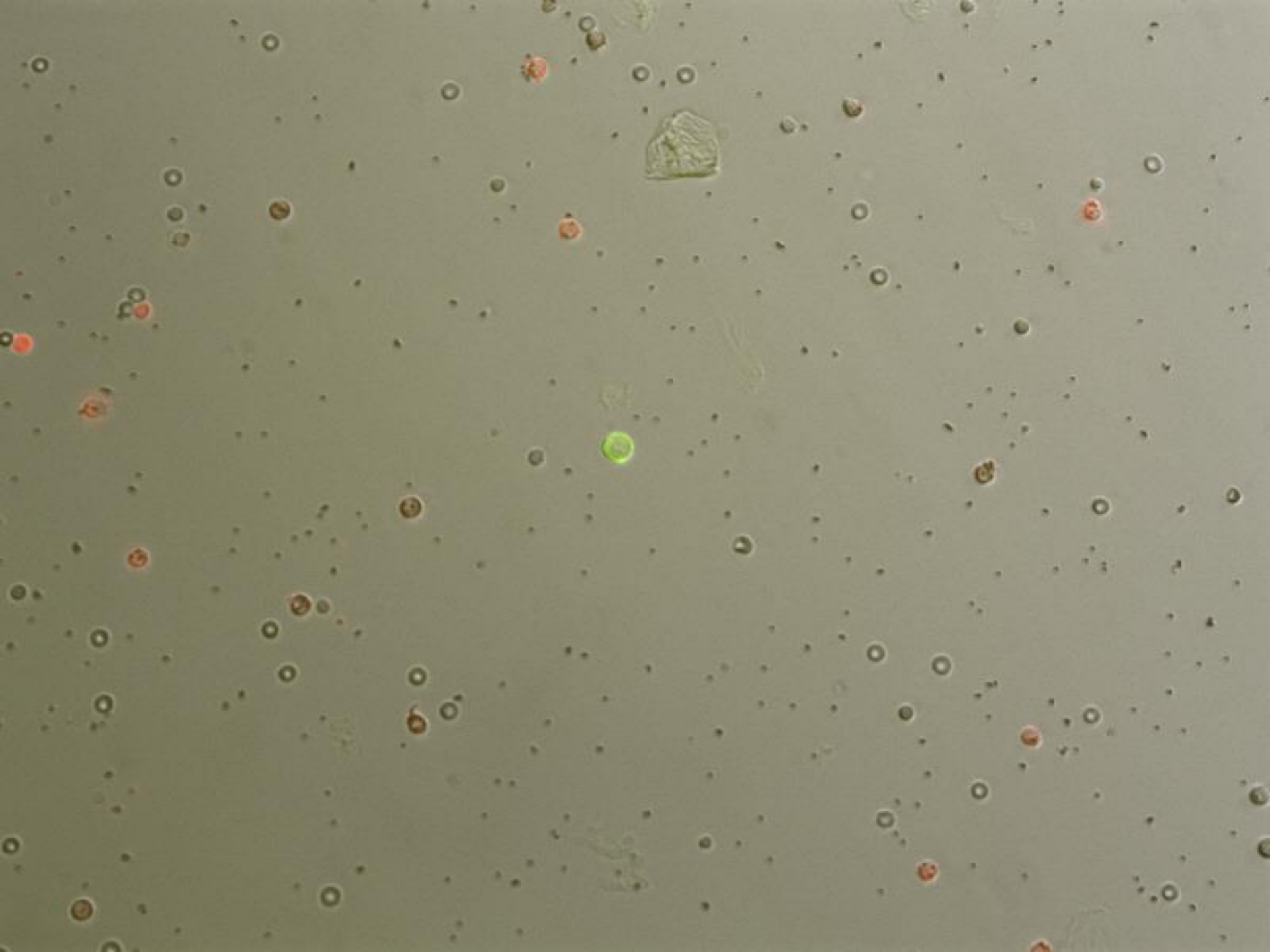
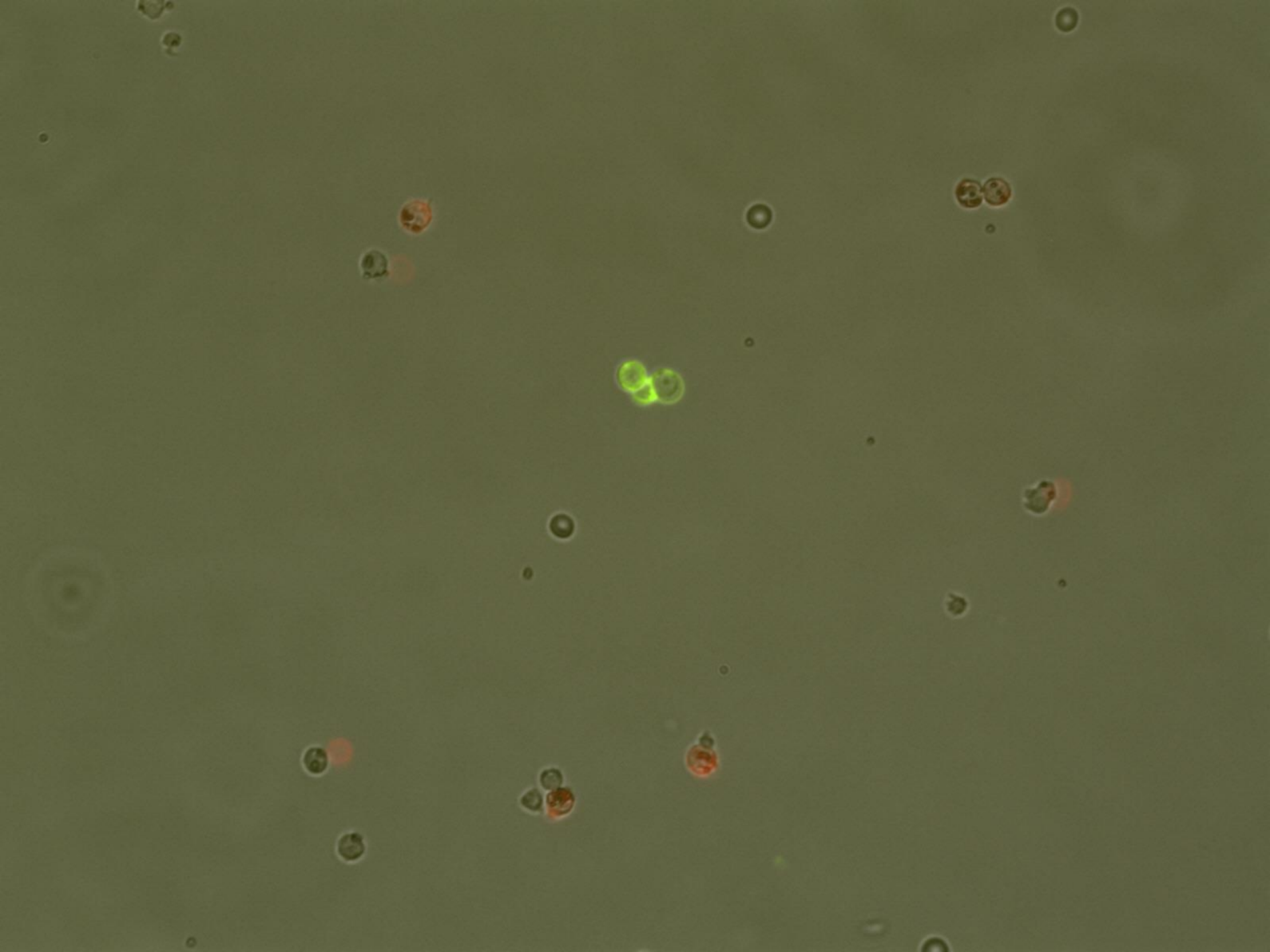
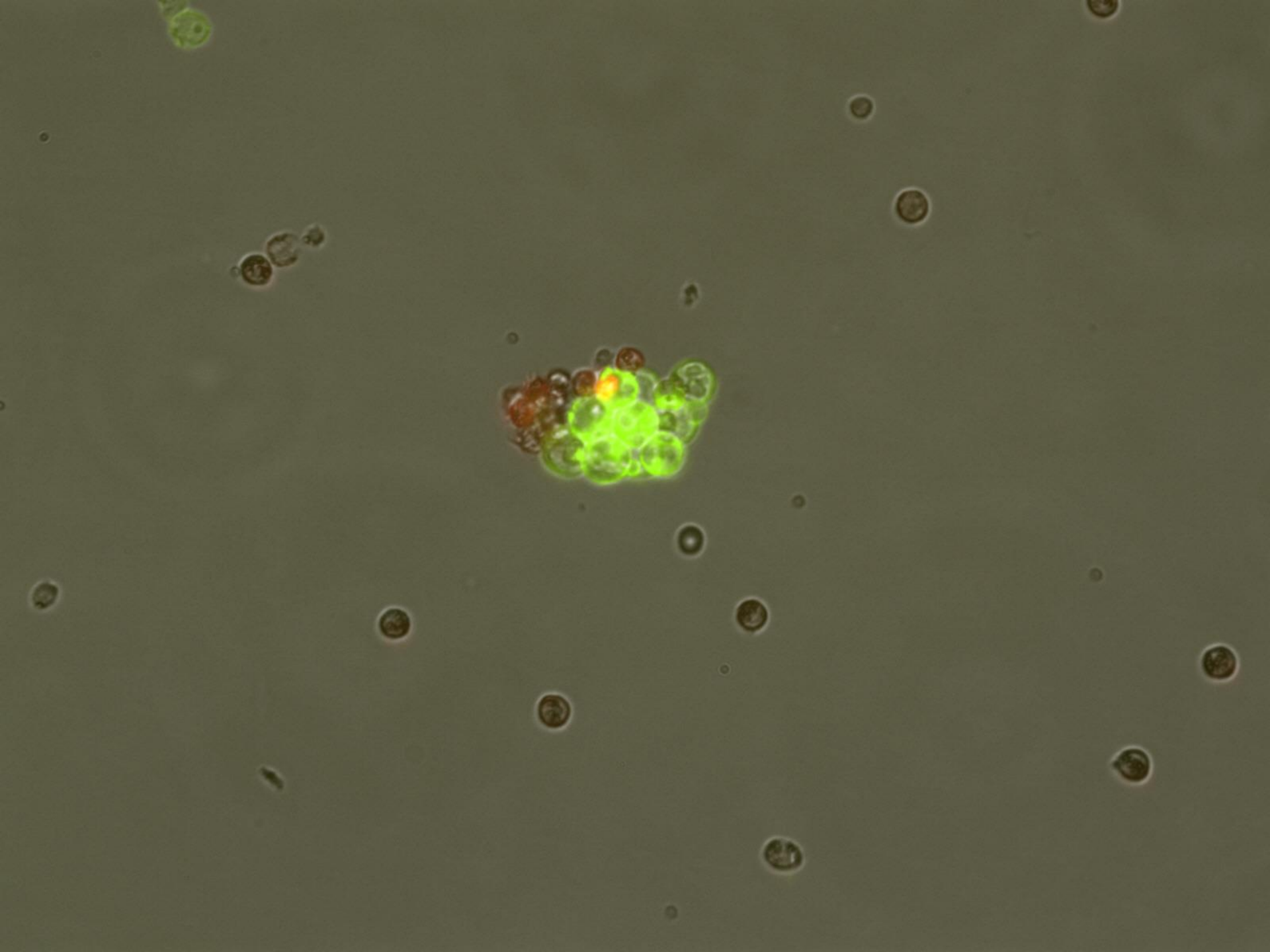
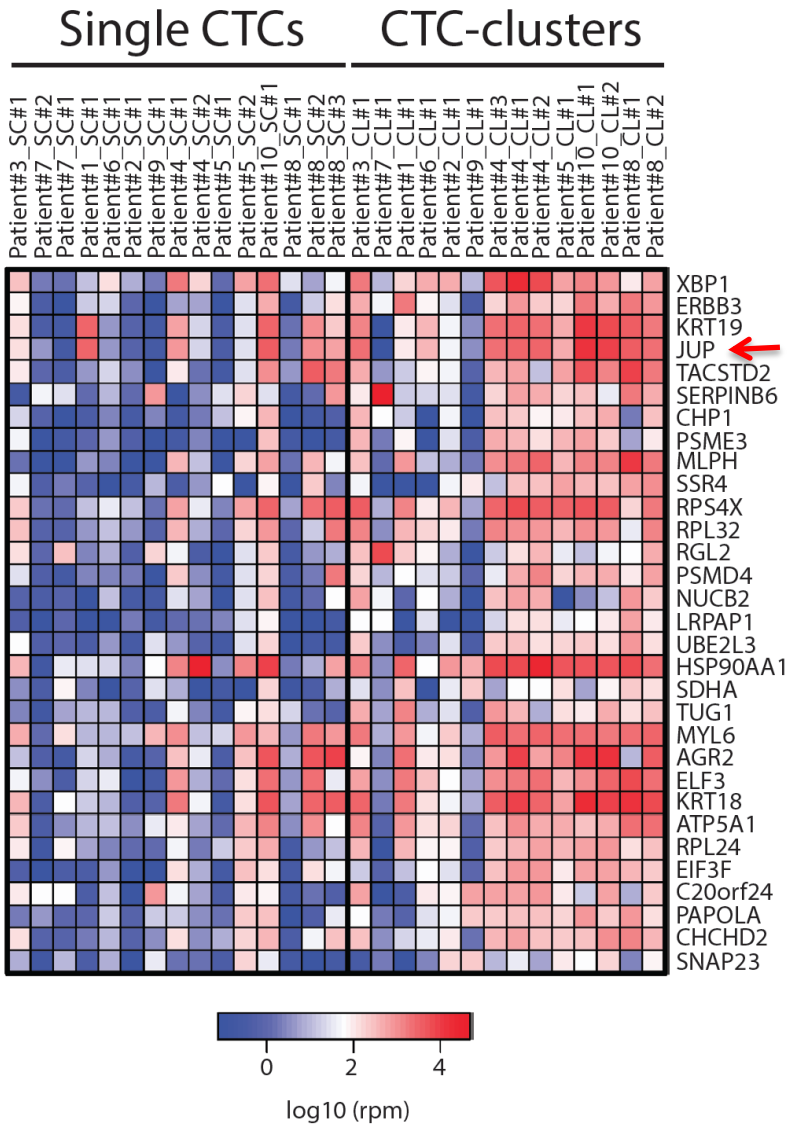


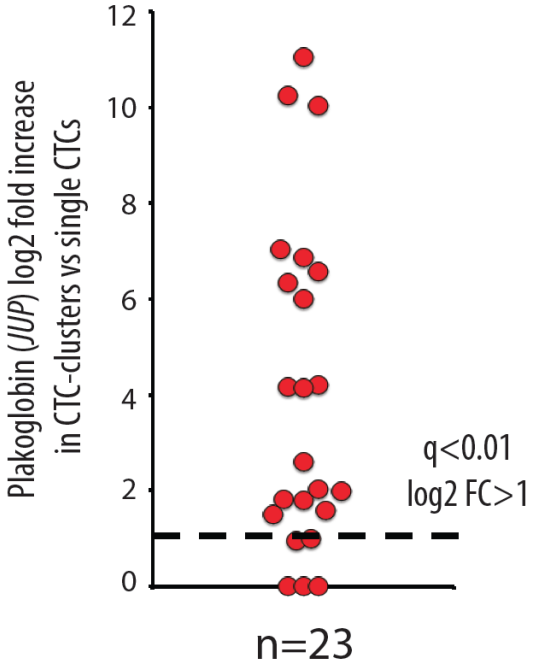




Single cell RNA sequencing of CTCs vs CTC-clusters

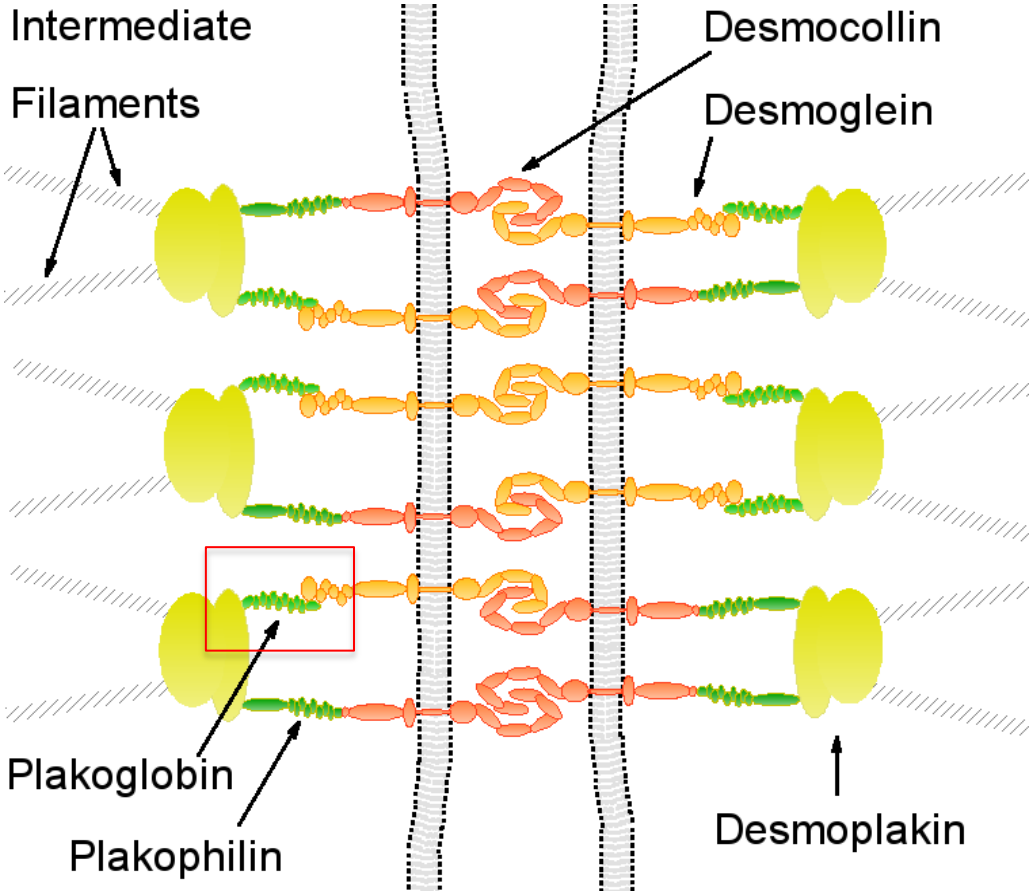


Plakoglobin increased ~215-fold in clusters *vs* Single CTCs



Aceto et al., ***Cell***, *2014*

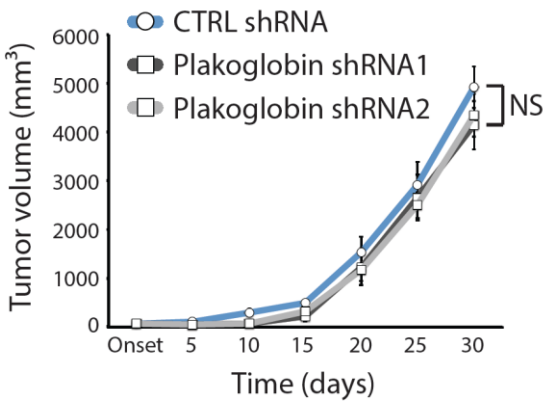
Plakoglobin as component of desmosomes (and adherence junctions)



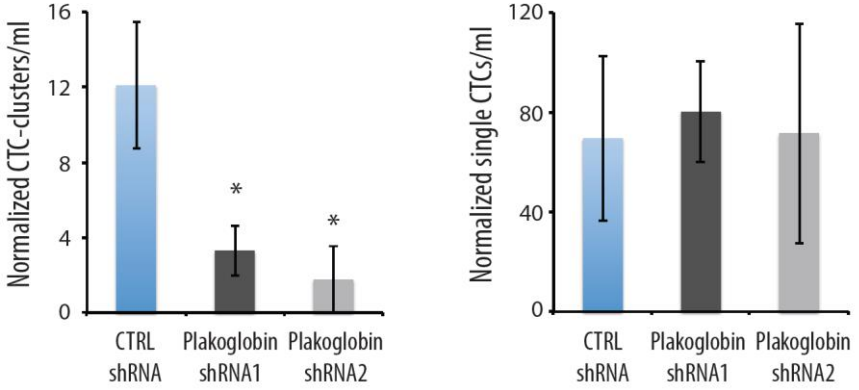
Targeting CTC-clusters

Targeting cell-cell junctions to disrupt CTC-clusters and reduce the metastatic spread of cancer

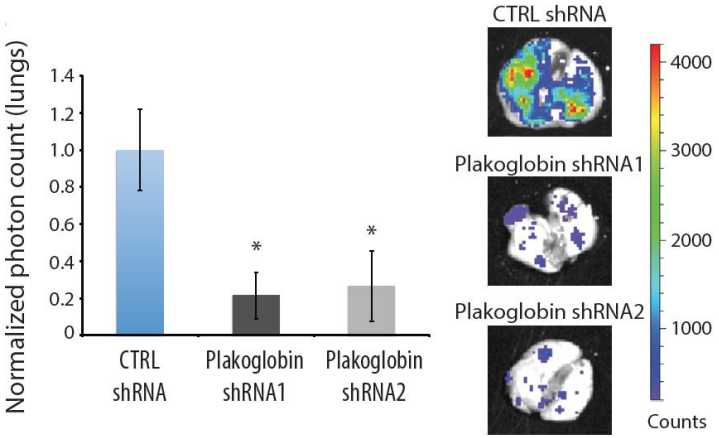
Plakoglobin knockdown in the primary tumor suppresses lung metastasis



No change in growth of primary tumor xenograft



Reduced CTC-clusters, but not single CTCs, from primary tumor xenograft



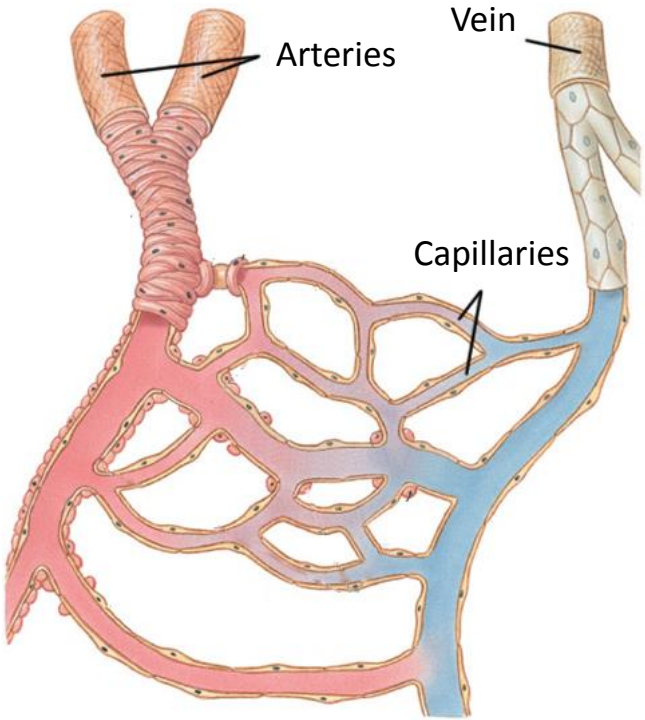
Reduced lung metastases from primary tumor xenograft

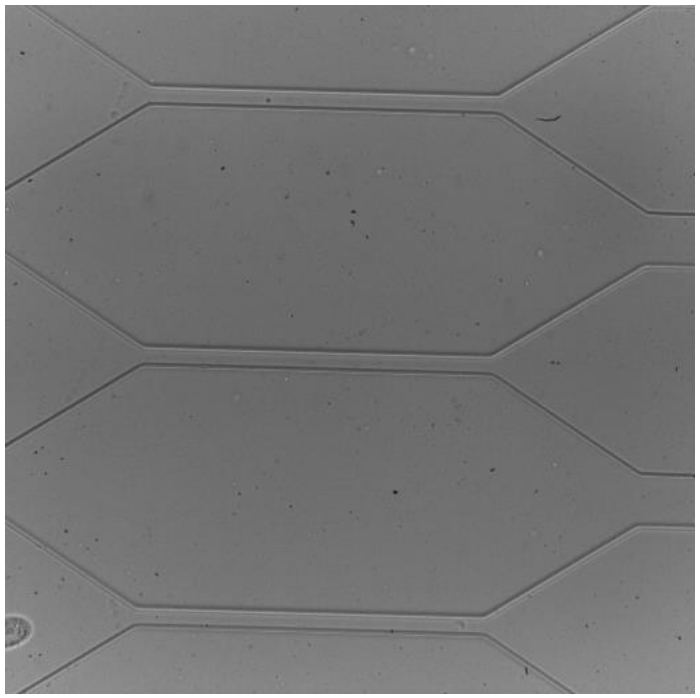
*Aceto et al., **Cell**, 2014*

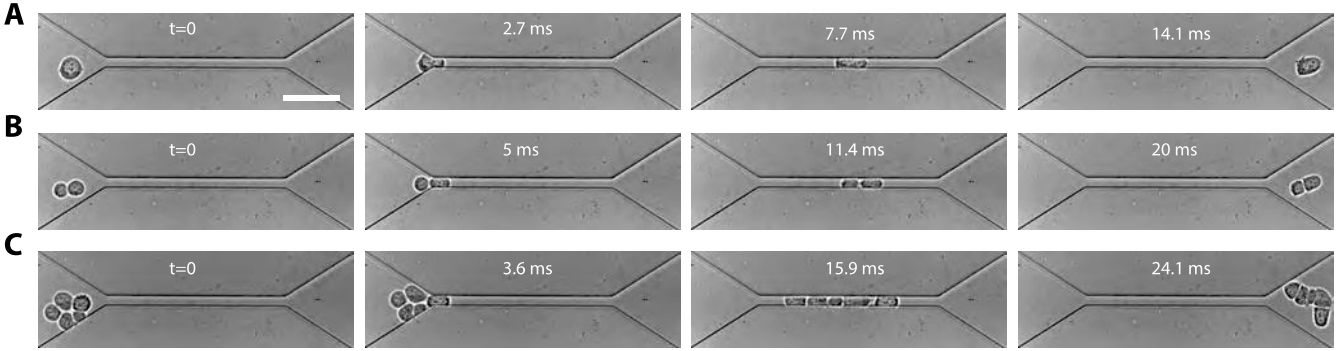
The Cluster-CHIP

Are we capturing all CTC-clusters?

Cluster Traverse Through Capillaries







Sarioglu, Aceto* et al.,*
***Nature Methods**, 2015*

Microfluidic Capture of CTCs

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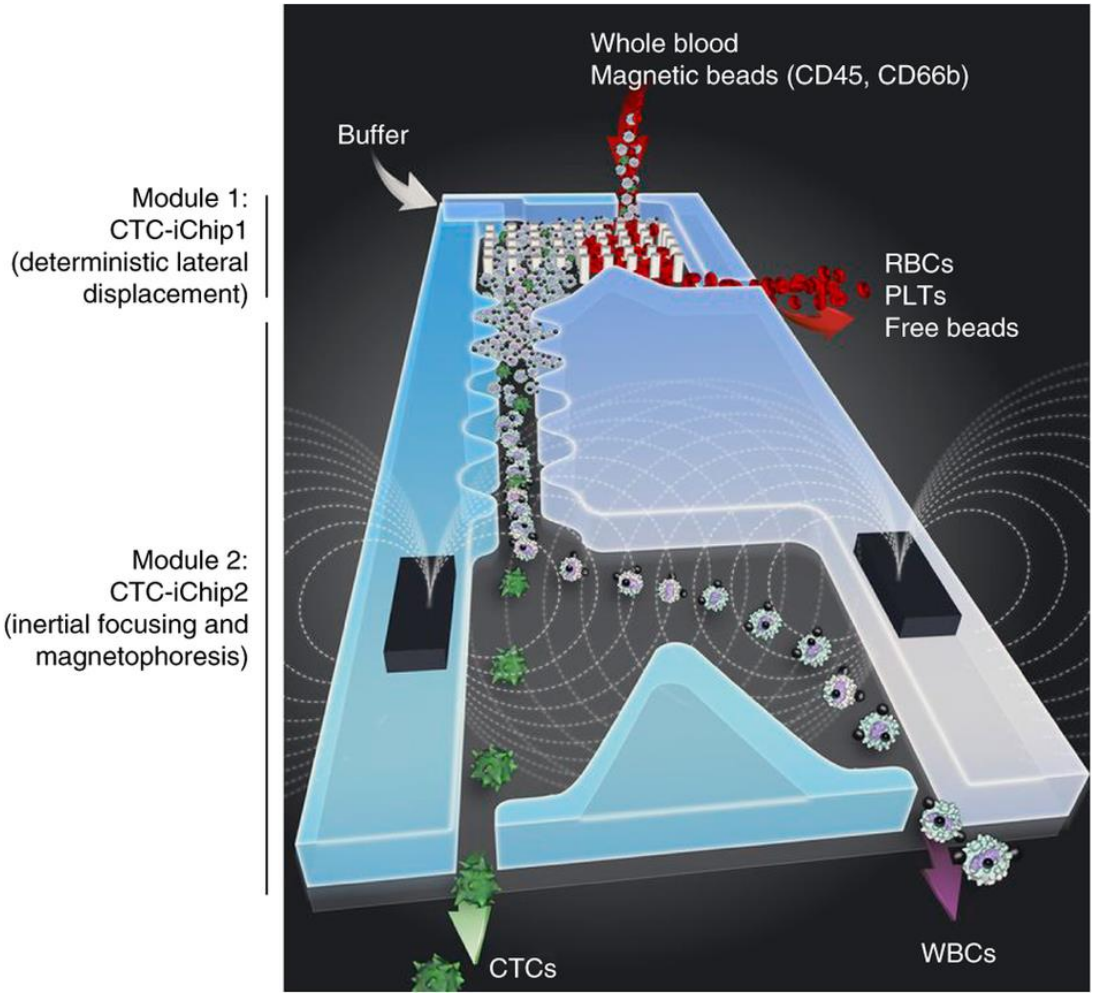

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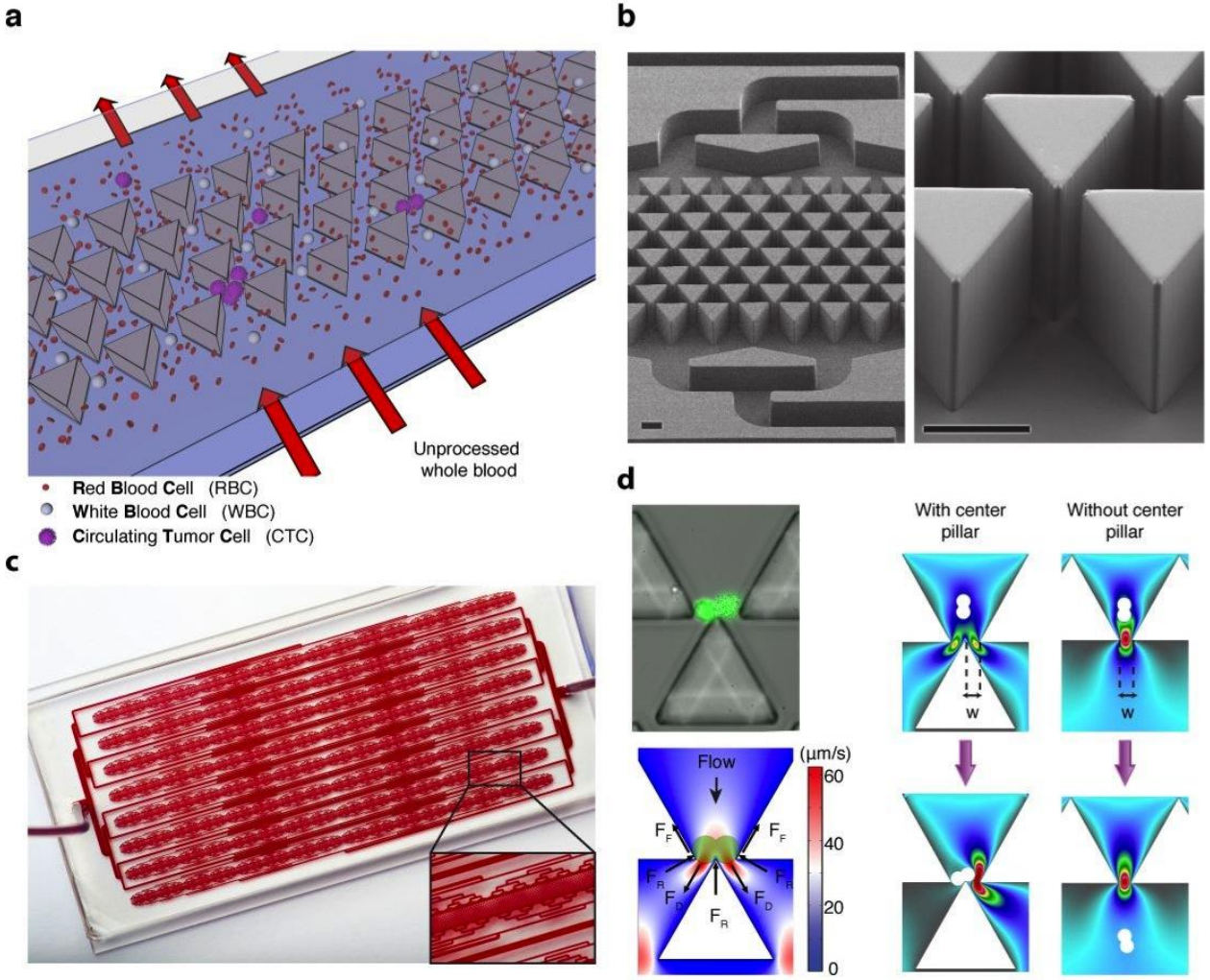
The Cluster-CHIP



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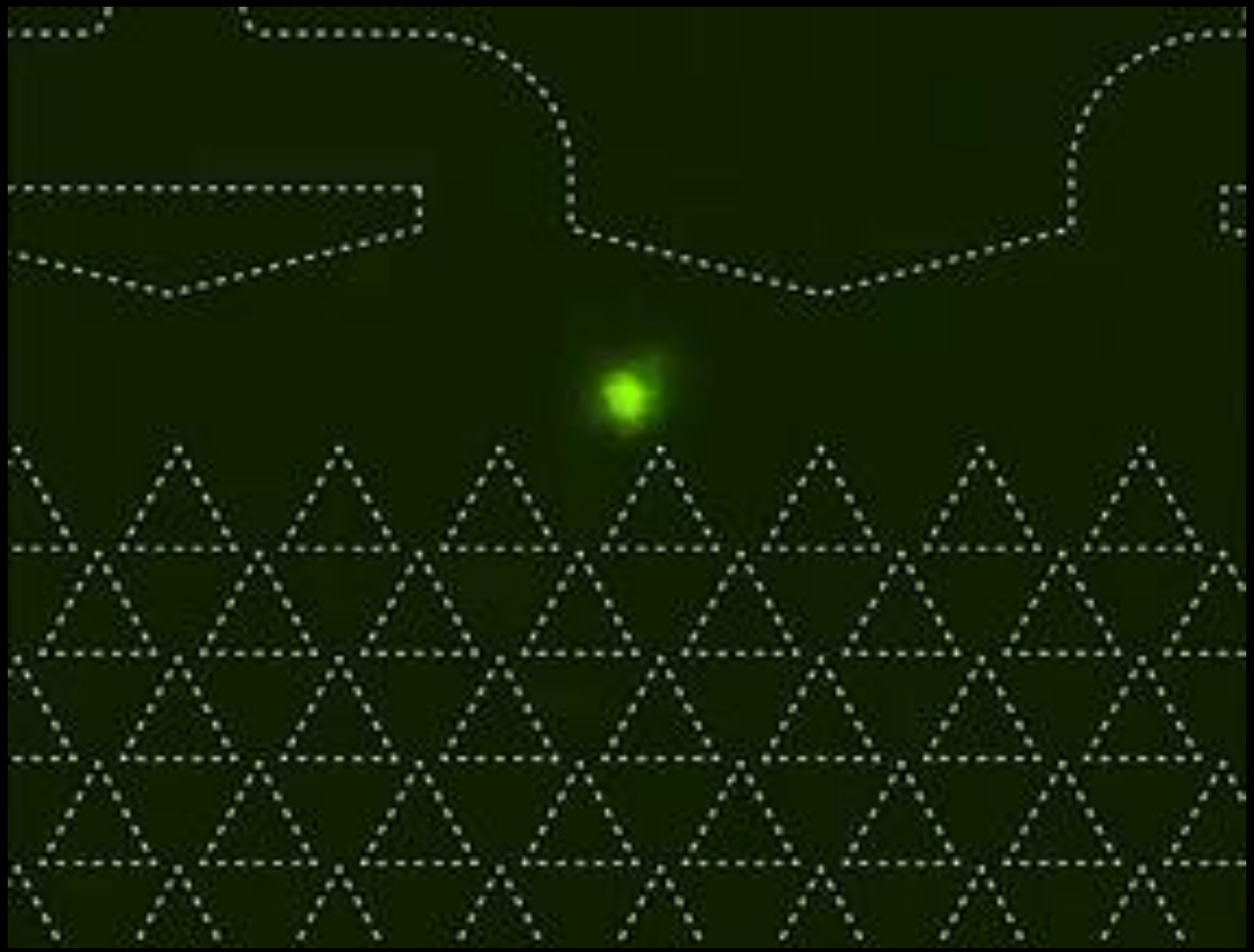
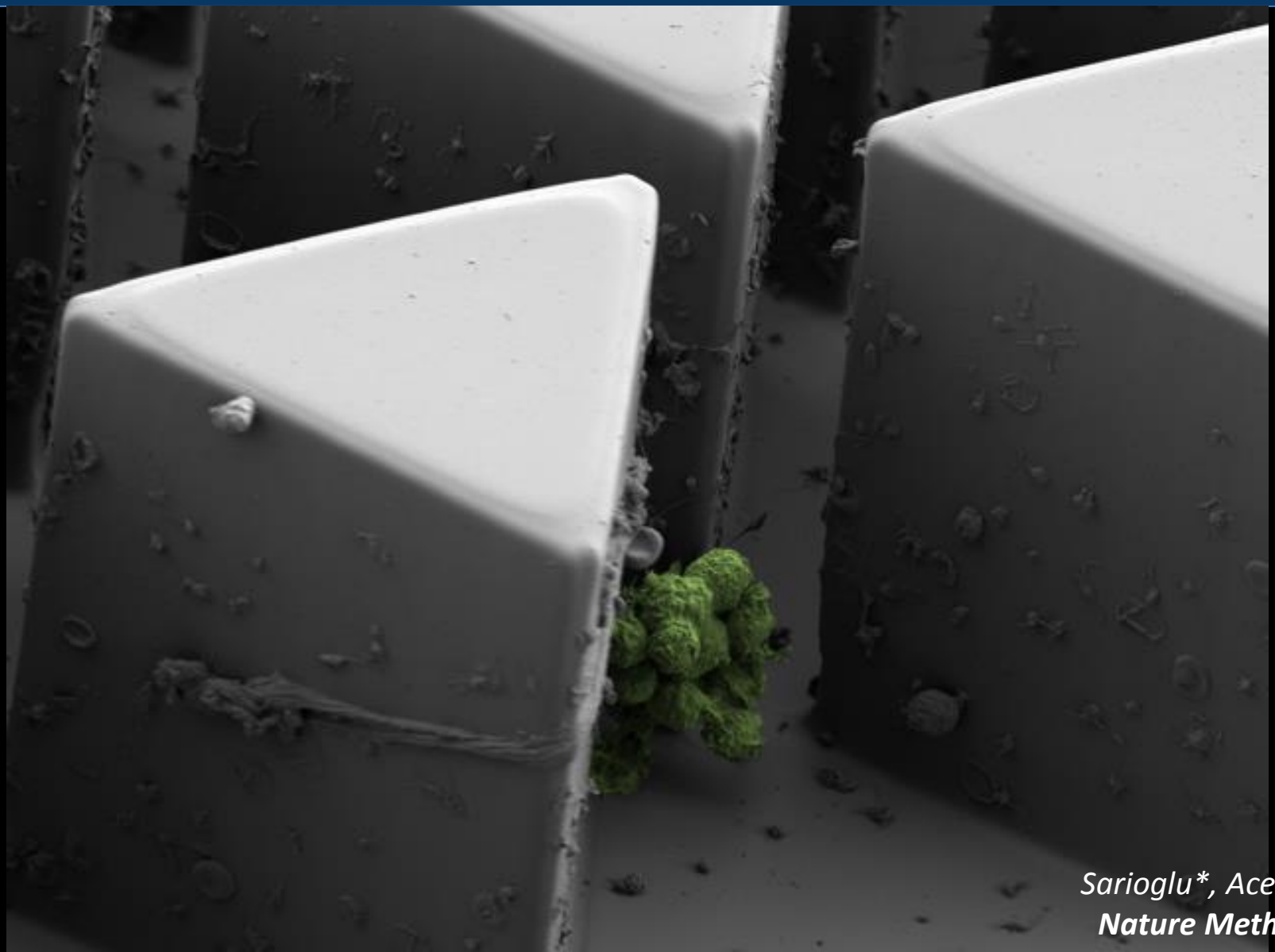


EM on the Cluster-CHIP

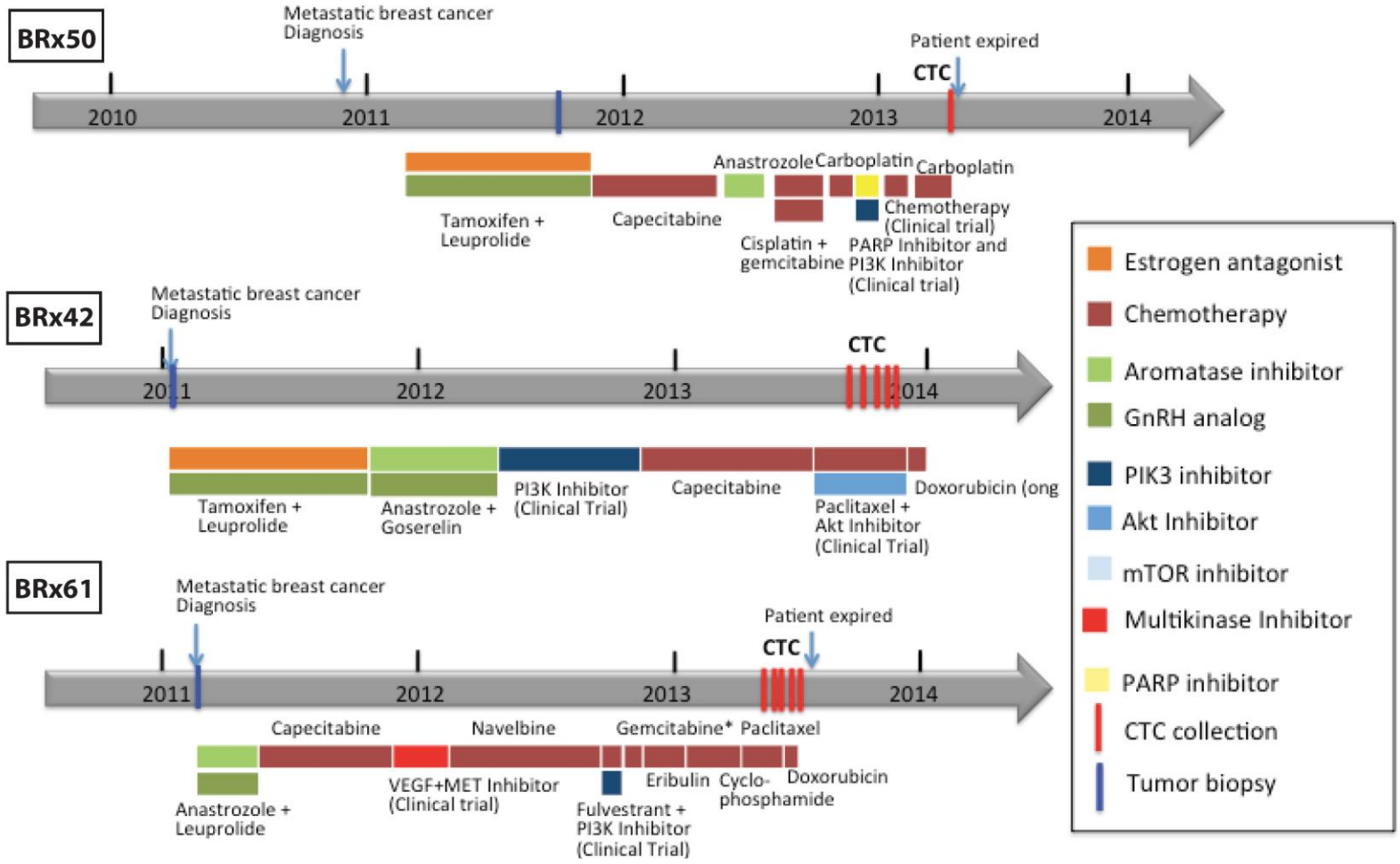


Sarioglu, Aceto* et al.,*
***Nature Methods**, 2014*

3. Ex vivo Culture of CTCs

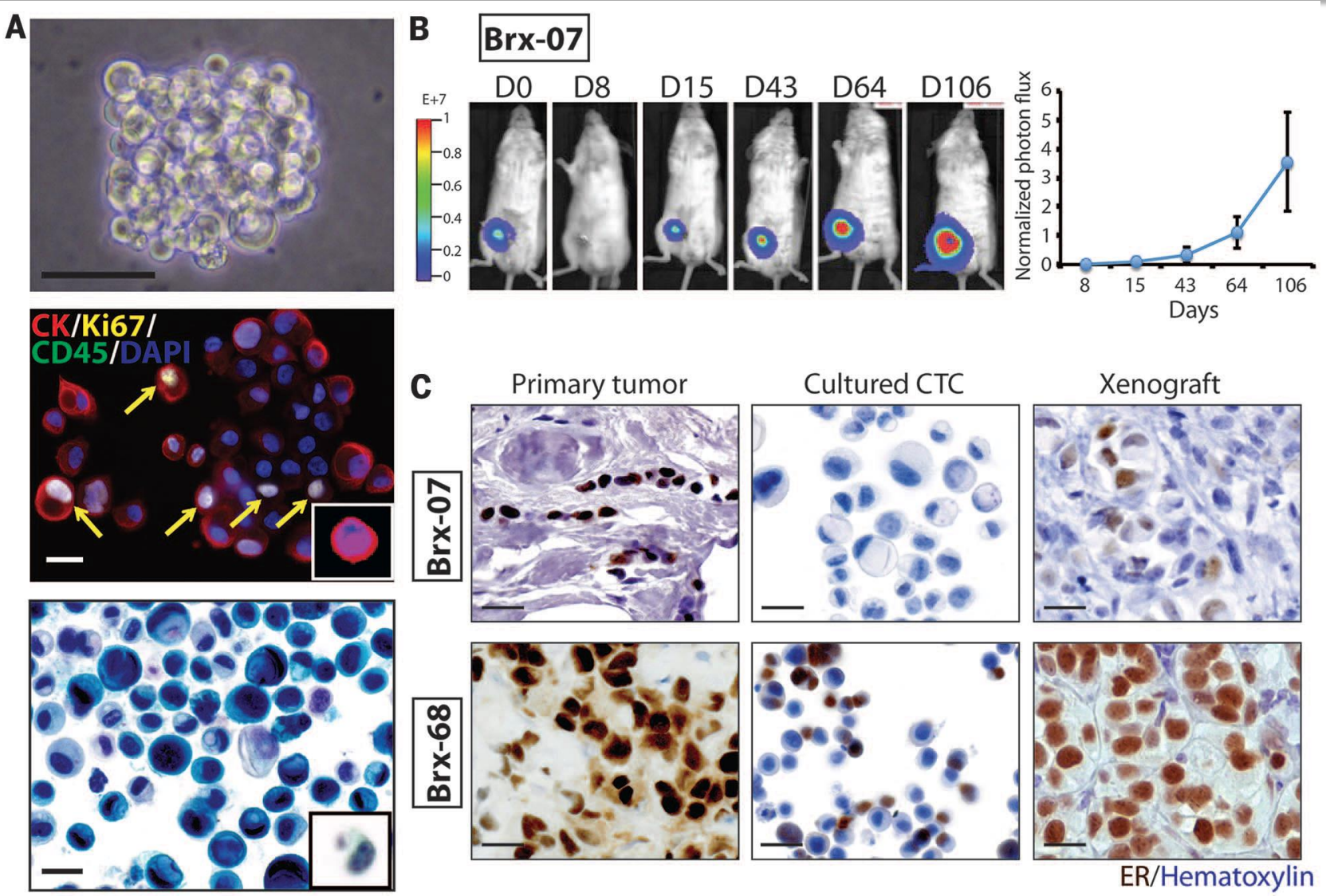
Can we use CTCs to non-invasively monitor drug susceptibility in patients?

Typical BC Patient History



*Yu, Bardia, Aceto et al., **Science**, 2014*

Ex vivo Culture of CTCs



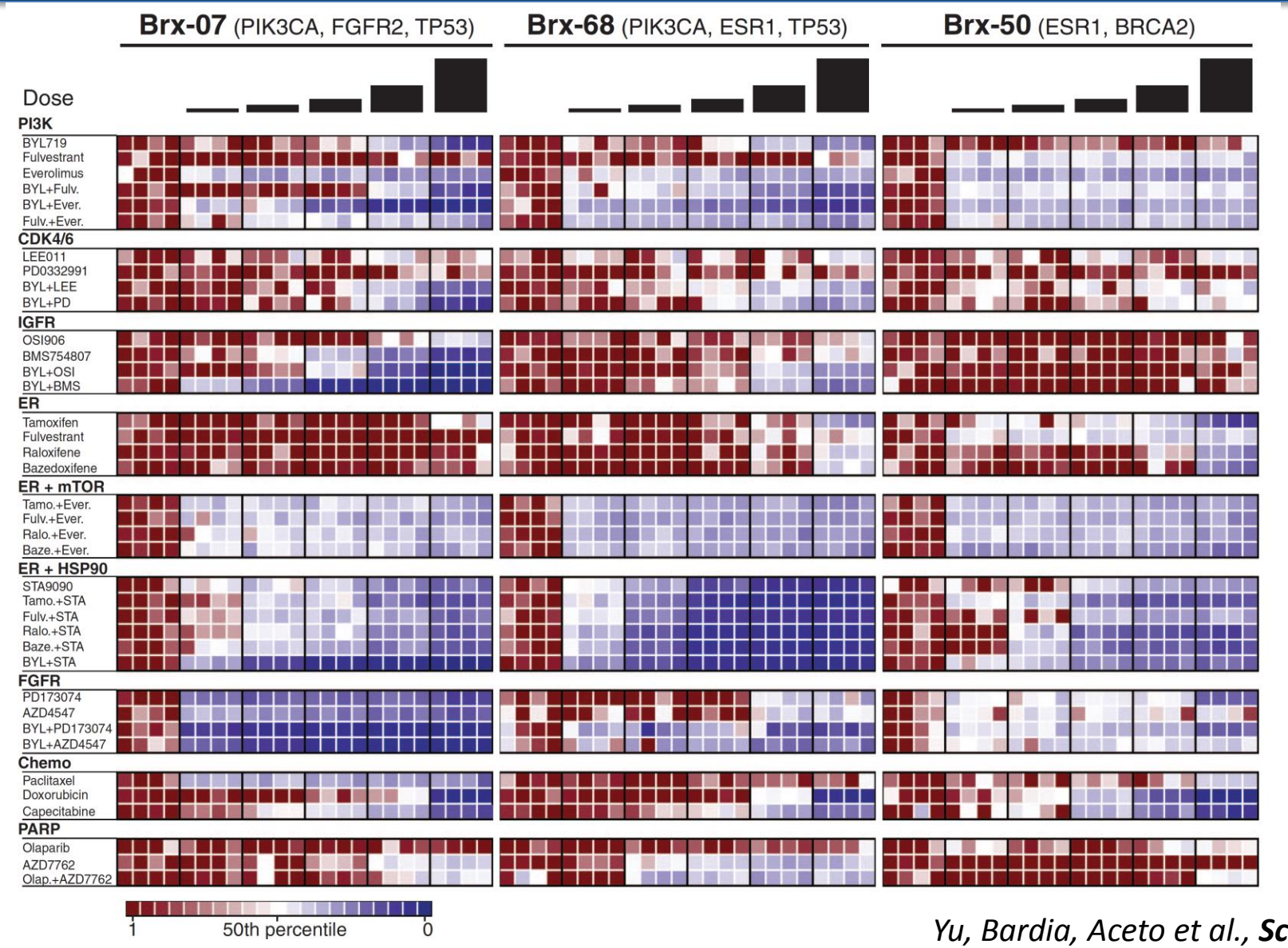
*Yu, Bardia, Aceto et al., **Science**, 2014*

Mutation Analysis

Case	Gene	DNA	Protein	Allele frequency†	In pretreatment tumor‡	In multiple CTC lines	Known mutation§
BRx33‖	ESR1	A1613G	D538G	0.24	–	–	Br,# En
	NUMA1	C5501T	S1834L	0.39	–	–	Br
BRx07‖	TP53	G853A	E285K	0.99	No	–	Bl, Br, Co, HN, Lu
	PIK3CA	A3140T	H1047L	1	No	–	Br, Co, GBM, HN, K_i, Lu, Me, Mel, Ov, En
	FGFR2	T1647A	N549K	0.46	No	–	Br, En
	CDH1	C790T	Q264*	1	Yes	–	Br
	APC	G7225A	G2409R	0.47	Yes	–	Mel
	DGKQ	G2530A	D844N	0.55	–	–	Lu
	MAML2	A2569G	M857V	0.52	–	–	Lu
BRx68	TP53	C1009T	R337C	0.99	No	Yes	Br, Co, HN, Hem, Ov
	ESR1	A1610C	Y537S	0.47	No	Yes	Br#, En
	PIK3CA	A3140G	H1047R	0.7	Yes	Yes	Br, Co, GBM, HN, K_i, Lu, Me, Mel, Ov, En
	MSN	G1153A	E385K	0.25	–	–	En
BRx50‖	ESR1	T1607C	L536P	0.06††	–	–	Br#
	IKZF1	G1444T	G482C	0.09	–	–	Hem
	BRCA2¶	T6262del	L2039fs	–	–	–	Br (germ line)
BRx42	PIK3CA	G3145C	G1049R	0.60	Yes	Yes	Br, En, K_i
	PIK3CA	C1097G	P366R	0.54	–	–	Br
	KRAS	G35T	G12V	0.99	No	Yes	Br, Co, Hem, Es, GBM, Lu, Ov, En
	IGF1R	G3613A	A1205T	0.06	–	–	Hem
BRx61	TP53	G610T	E204*	0.98	No	Yes	Bl, Br, K_i, Lu, Ov

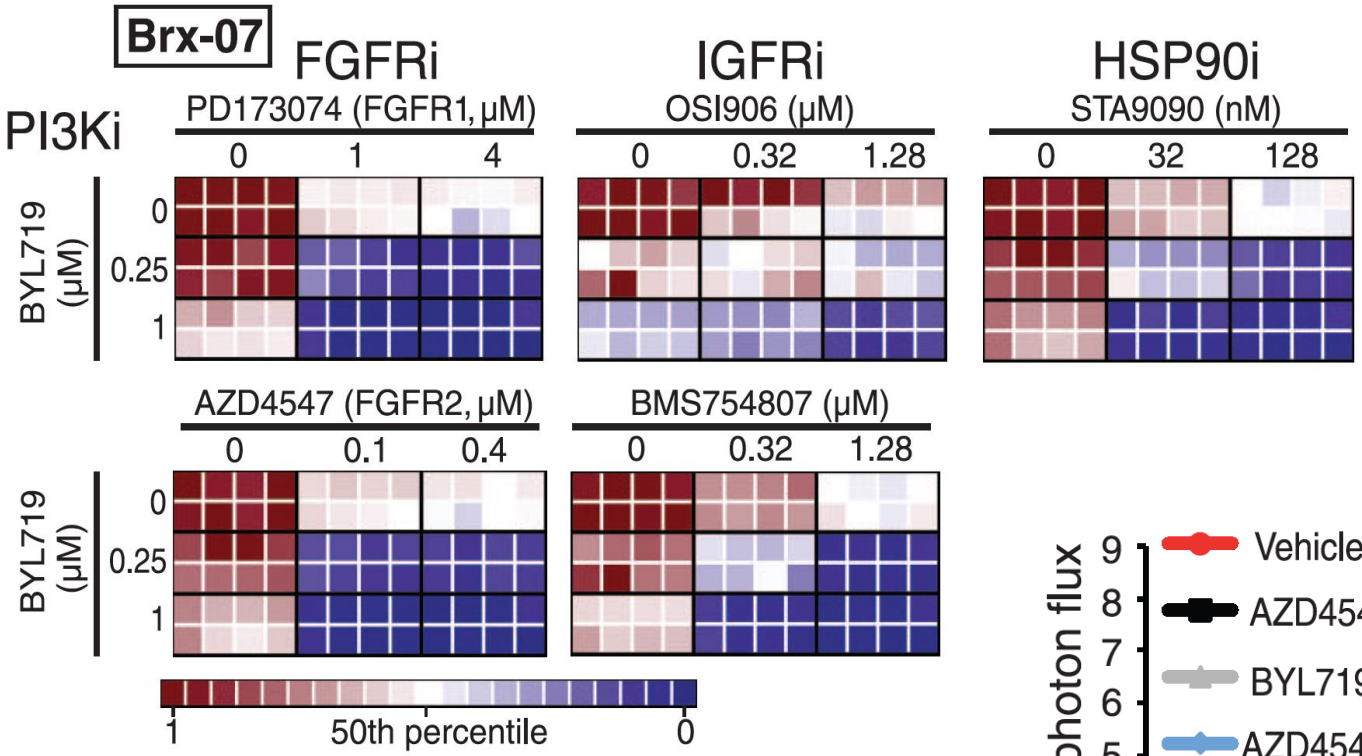
*Yu, Bardia, Aceto et al., **Science**, 2014*

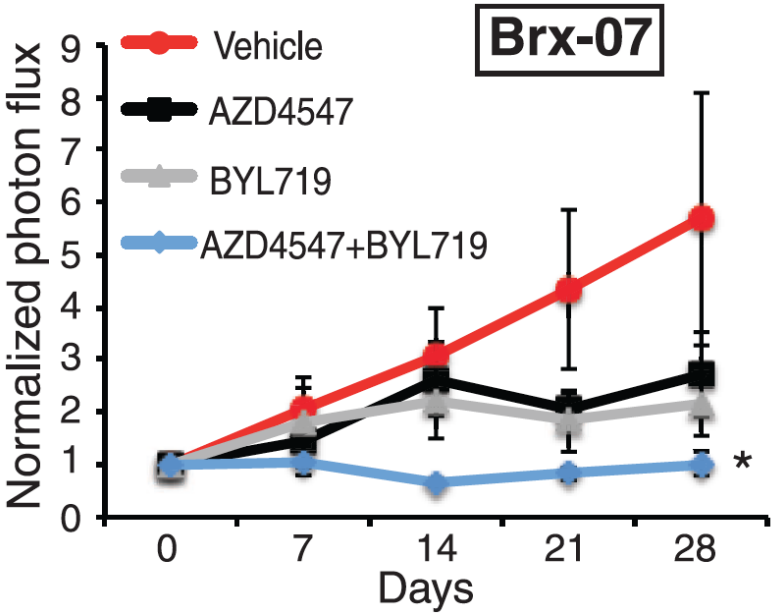
Mutation-based screening to identify drug susceptibility



Yu, Bardia, Aceto et al., ***Science****, 2014*

Validation of the best drug combinations in mouse models





Yu, Bardia, Aceto et al.
***Science**, 2014*

Take home

1. Analysis of CTCs can be a powerful tool to dissect **mechanisms of cancer metastasis** and noninvasively monitor evolving **drug susceptibility** in patients

1. **Microfluidics devices** are required to isolate CTCs from blood samples derived from patients with cancer

2. Compared to single migratory CTCs, **CTC-clusters are *rare but highly metastasis-competent***, and **targeting cell-cell junctions** may represent a valuable strategy to reduce metastatic spread

3. Culturing CTCs may help identify the **best therapies for individual cancer patients** over the course of their disease

4. What's next

The biology of CTC-clusters:

How/why/when are they generated?

What are their key signaling networks?

How can we suppress them in patients?

Acknowledgments

Cancer Metastasis Lab Basel
Sofia Gkountela
Barbara Szczerba
Cinzia Donato
Edward Richards
Ilona Krol

University Hospital Basel
Christoph Rochlitz
Alfred Zippelius
Walter Weber
Marcus Vetter
Julia Landin

ETH Zürich
Niko Beerenwinkel
Christian Beisel

Funding

Haber lab MGH/Harvard
Daniel Haber
Shyamala Maheswaran
David Miyamoto
Michael Rothenberg
Tilak Sunderasan
David Ting
Min Yu
Eric Zheng

Toner lab MGH/MIT/ Harvard
Mehmet Toner
Shannon Stott
Ravi Kapur
Fatih Sarioglu
Emre Ozkumur
Philipp Spuhler
John Walsh

Funding

Clinical MGH/Harvard
Lecia Sequist
Aditya Bardia
Elena Brechtel
David Louis
Dennis Sgroi
Andrew Chi
Doug Dahl
Keith Flaherty
Caitlin Koris
Donald Lawrence

Ramaswamy lab MGH/ Harvard
Sridhar Ramaswamy
Toshi Shioda
Ben Wittner