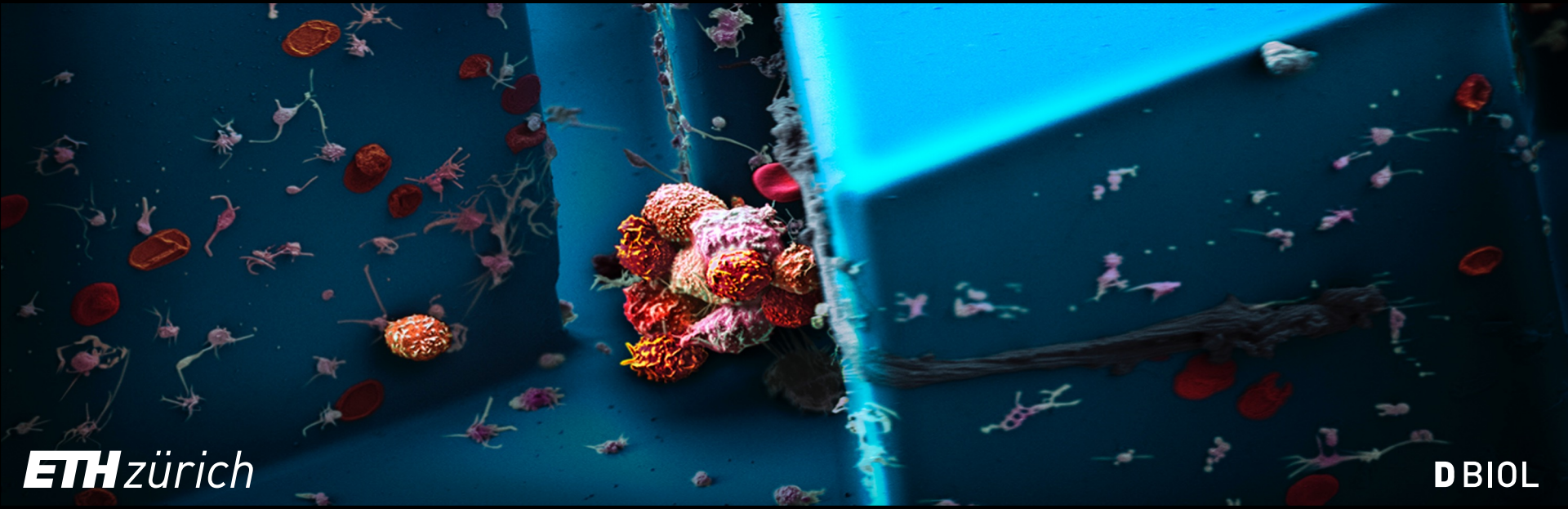
“Circulating Tumor Cells”

@ ProBio Symposium, Univesitätsspital Basel, 13 January 2023



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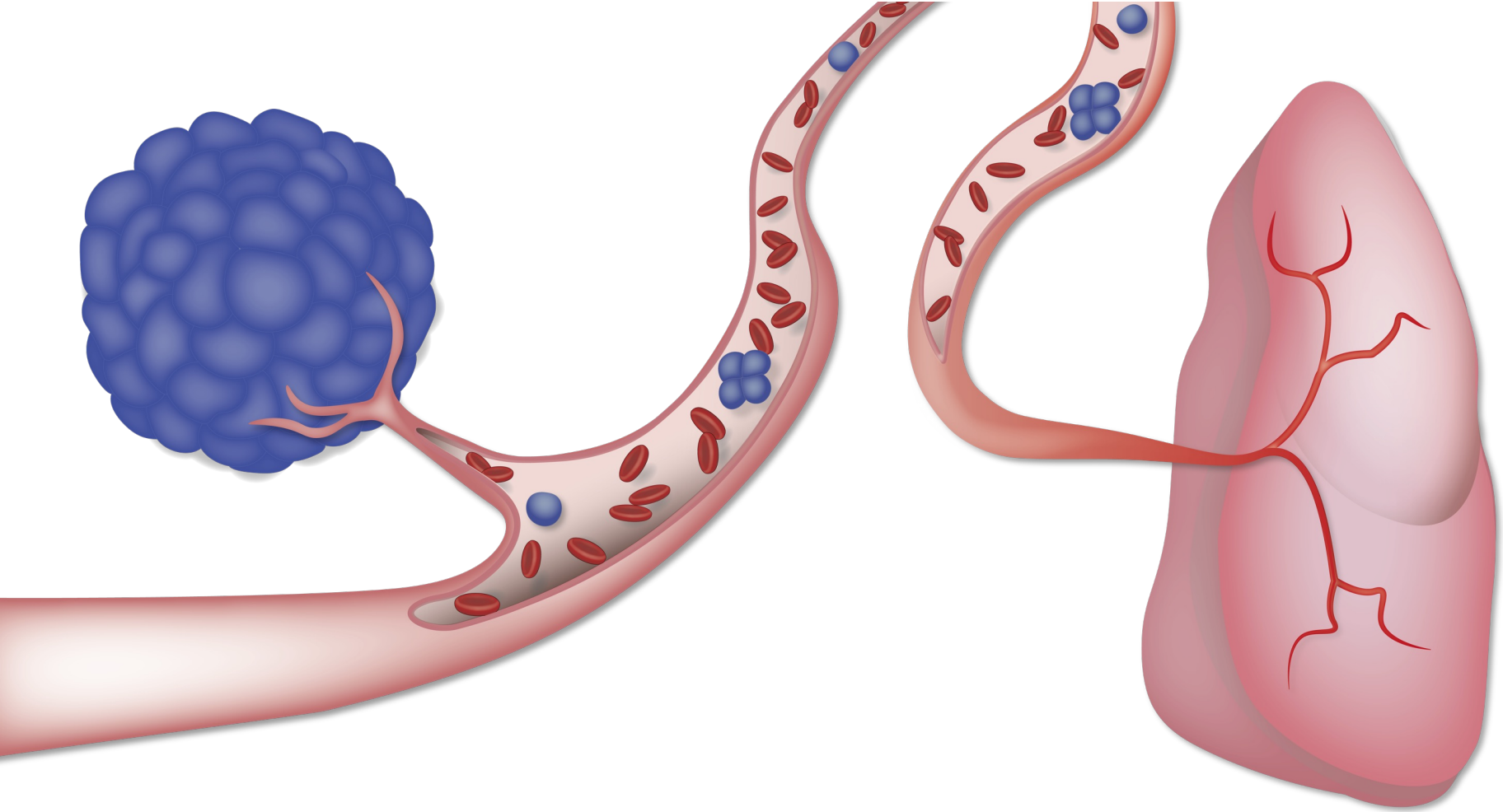
Declaration of Conflict of Interest

Co-founder, CSO and member of the Board:	PAGE Therapeutics AG
Honoraria, consultation fees and/or research support:	Novartis, Lilly, Pfizer, Swiss Re Group, ThermoFisher, Bracco Suisse, Basilea Pharmaceutica Ltd., Tethis S.p.A., Angle plc
Shareholder:	Novartis, PAGE Therapeutics AG



Presentation Outline

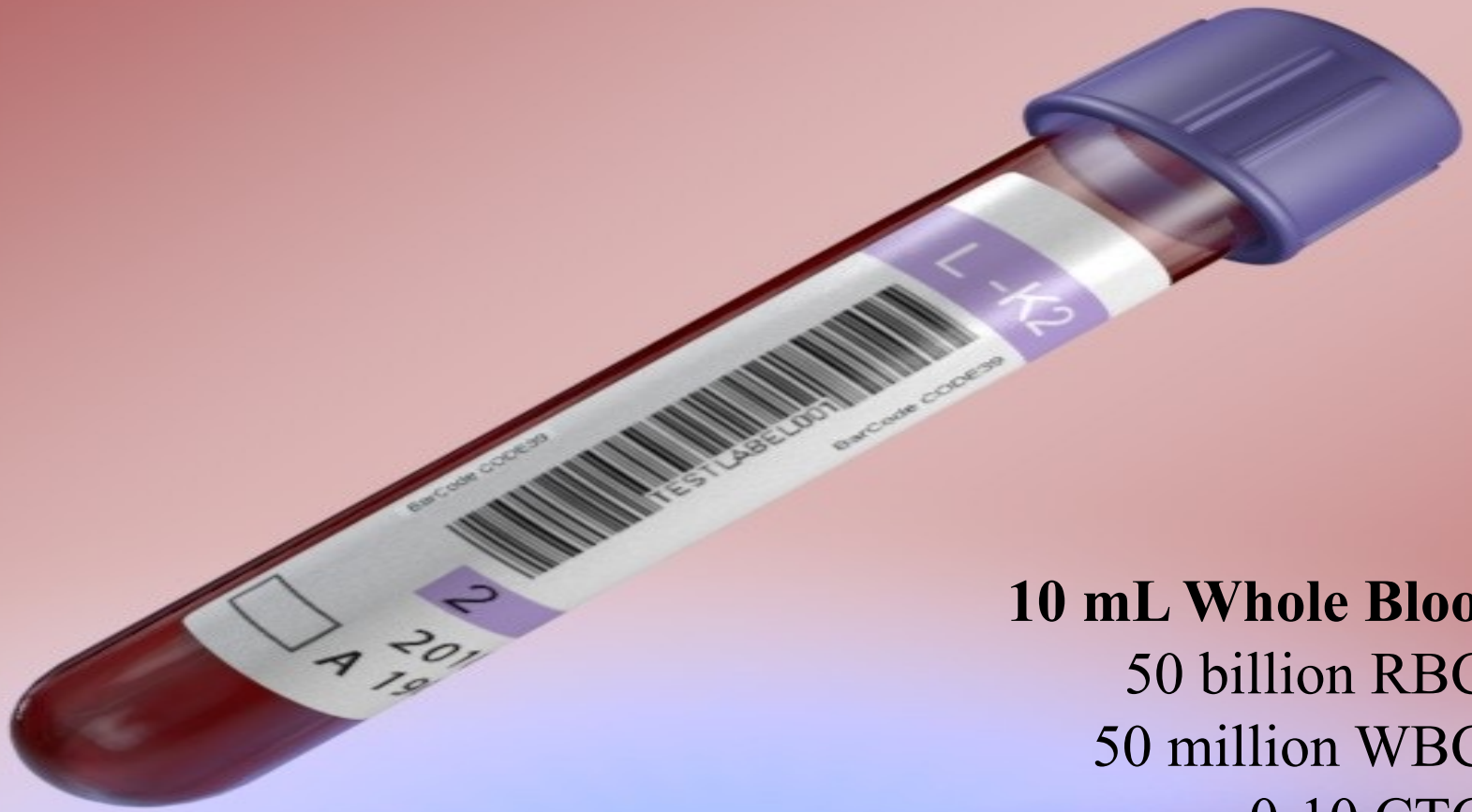
1) ***CTC capture***

2) ***Why focus*** *on CTC clusters*

3) ***Biology and Vulnerabilities*** *of CTC clusters*

4) ***Prostate CTC clusters***

CTC capture



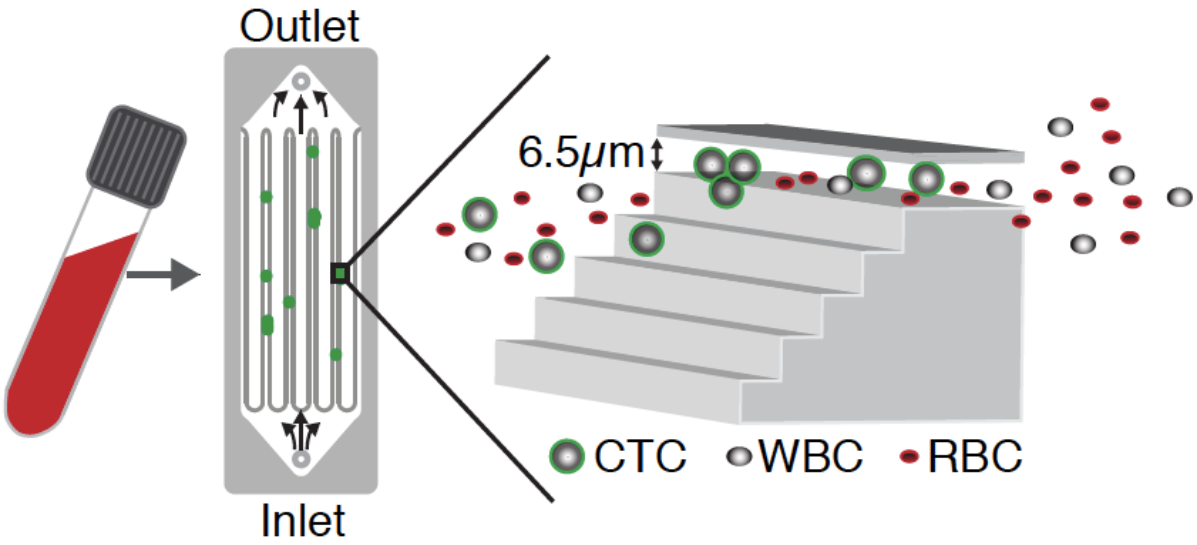
10 mL Whole Blood

50 billion RBCs

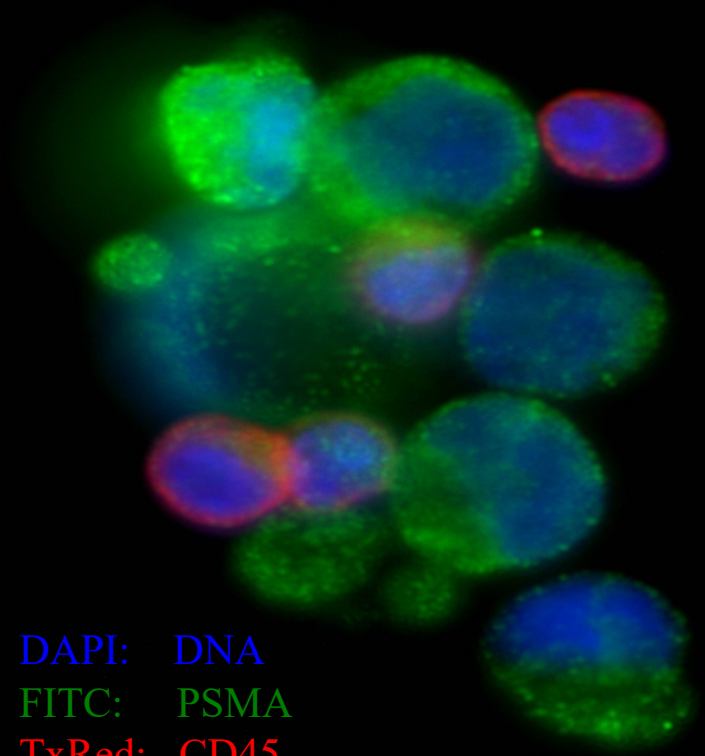
50 million WBCs

0-10 CTCs

Size-based, Antigen-independent CTC capture

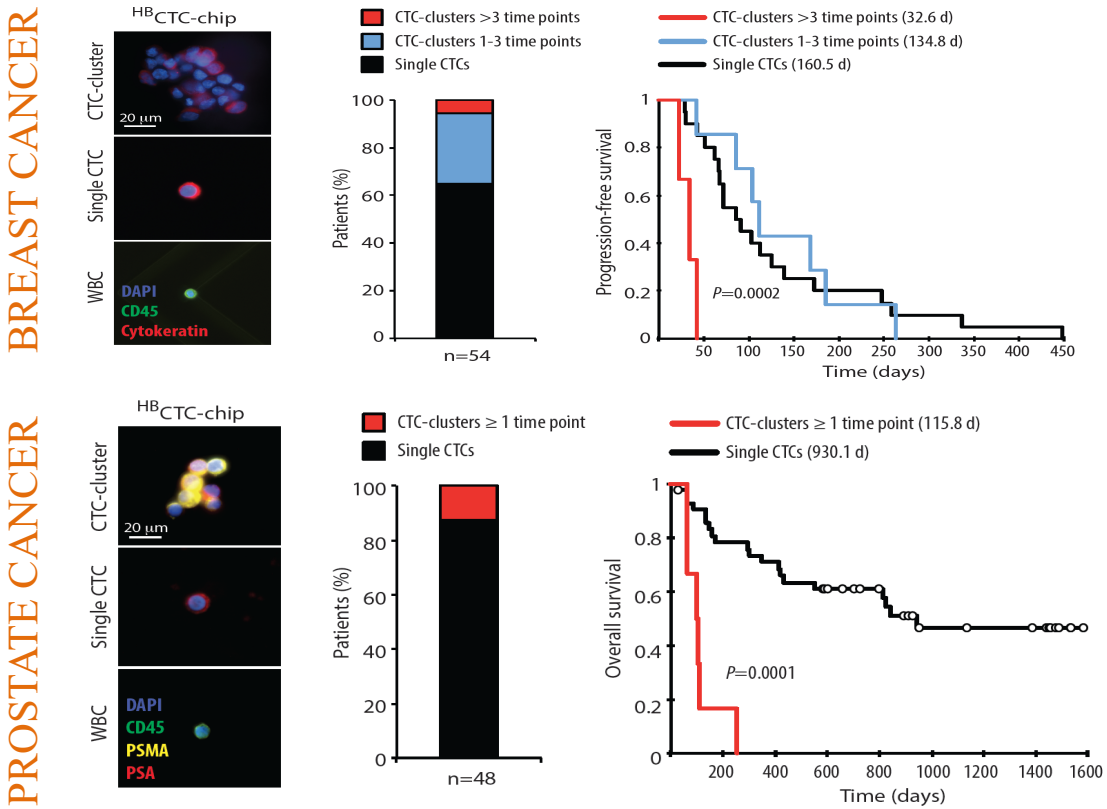


Why focus on CTC clusters?



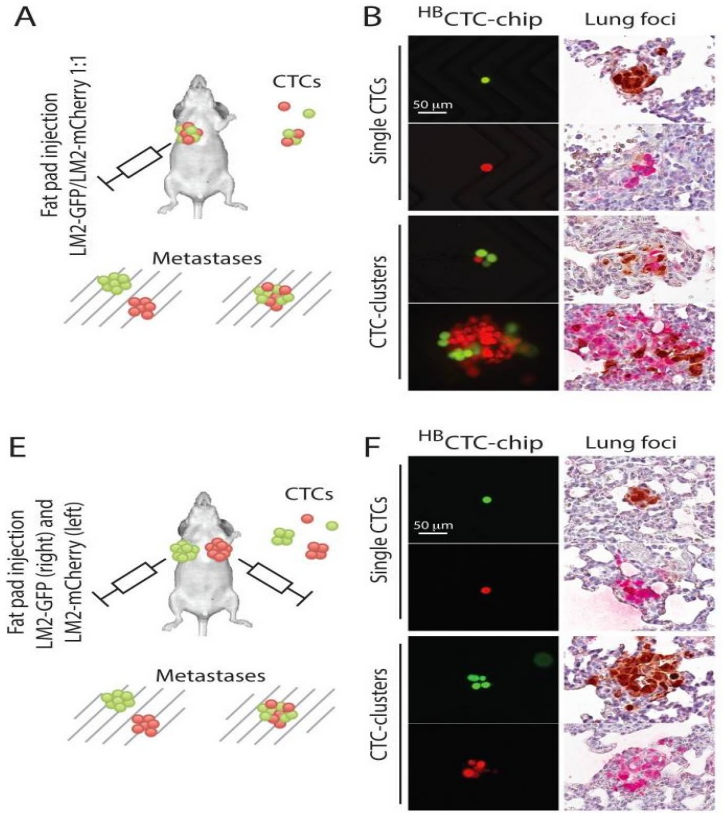
Stott et al., **Science Transl Med**, 2010

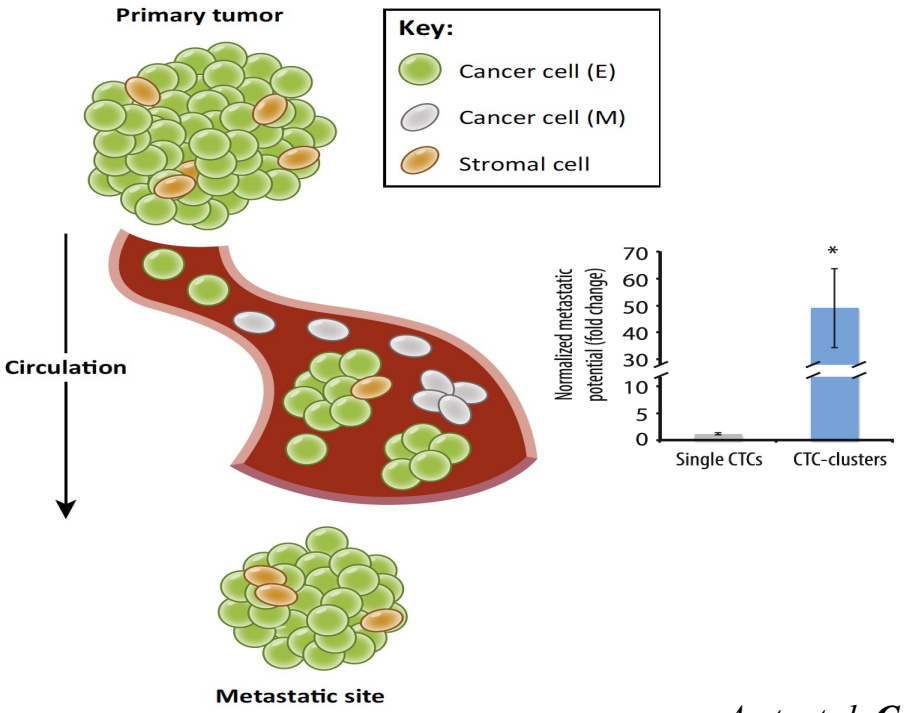
CTC clusters and poor prognosis in cancer patients



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

CTC clusters are Oligoclonal and 50x more metastatic than single CTCs





Aceto et al., ***Cell****, 2014*
Sarioglu, Aceto* et al.,* ***Nature Methods****, 2015*
Aceto et al., ***Trends in Cancer****, 2015*

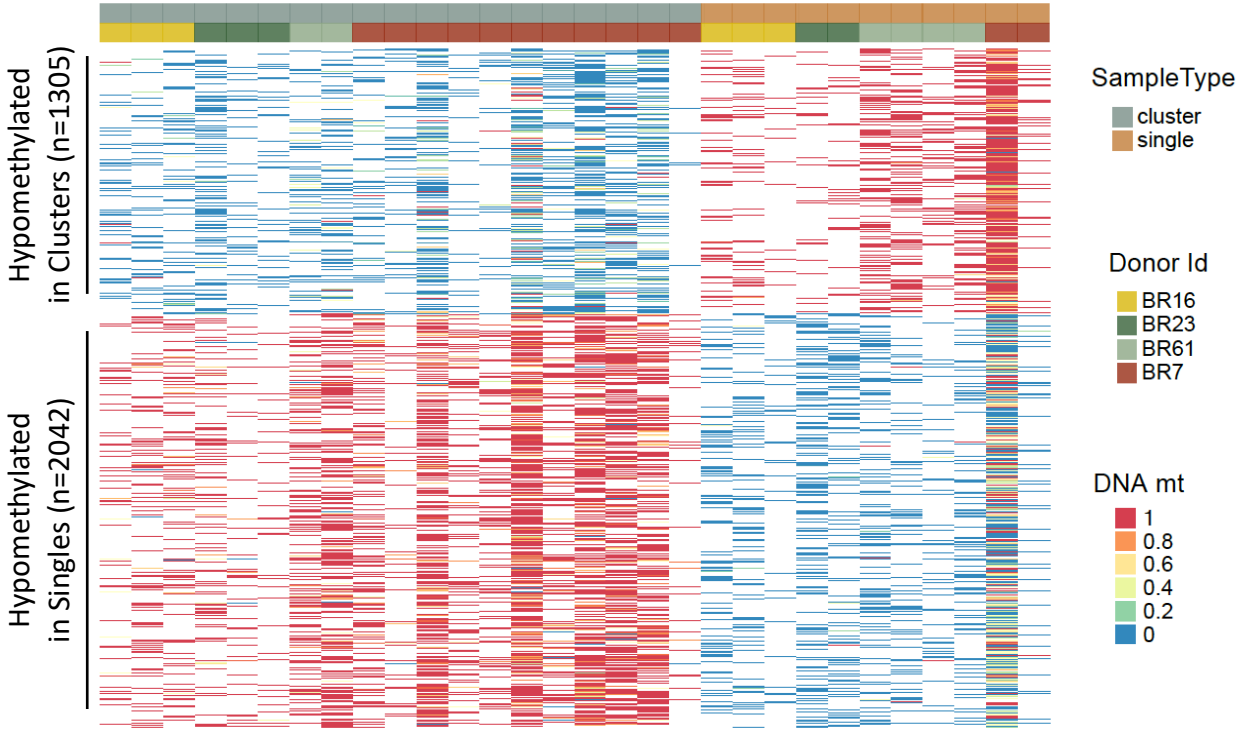
Biology and Vulnerabilities of CTC clusters

DNA methylation profile of single CTCs and CTC clusters

DMRs: 5 Kb regions with 80% methylation difference

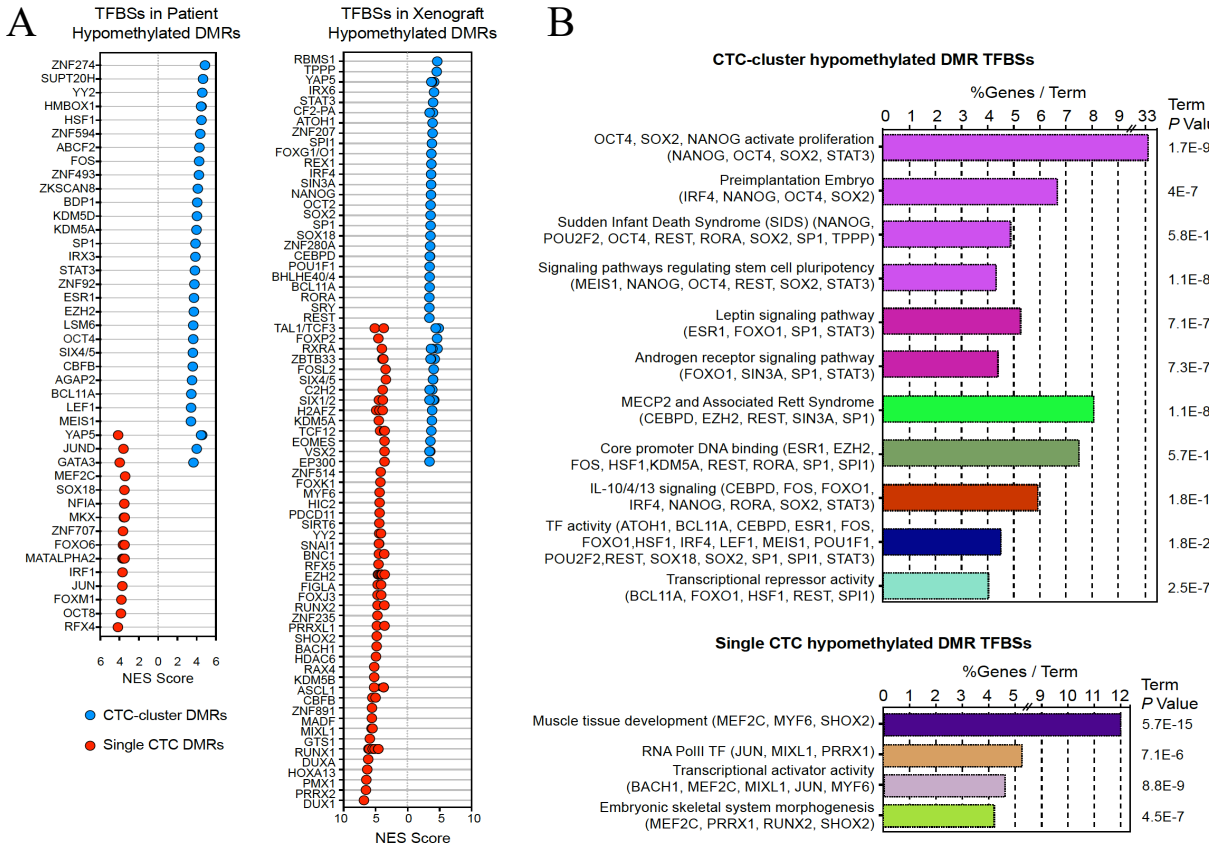
CTC Cluster-specific active networks

Single CTC-specific active networks



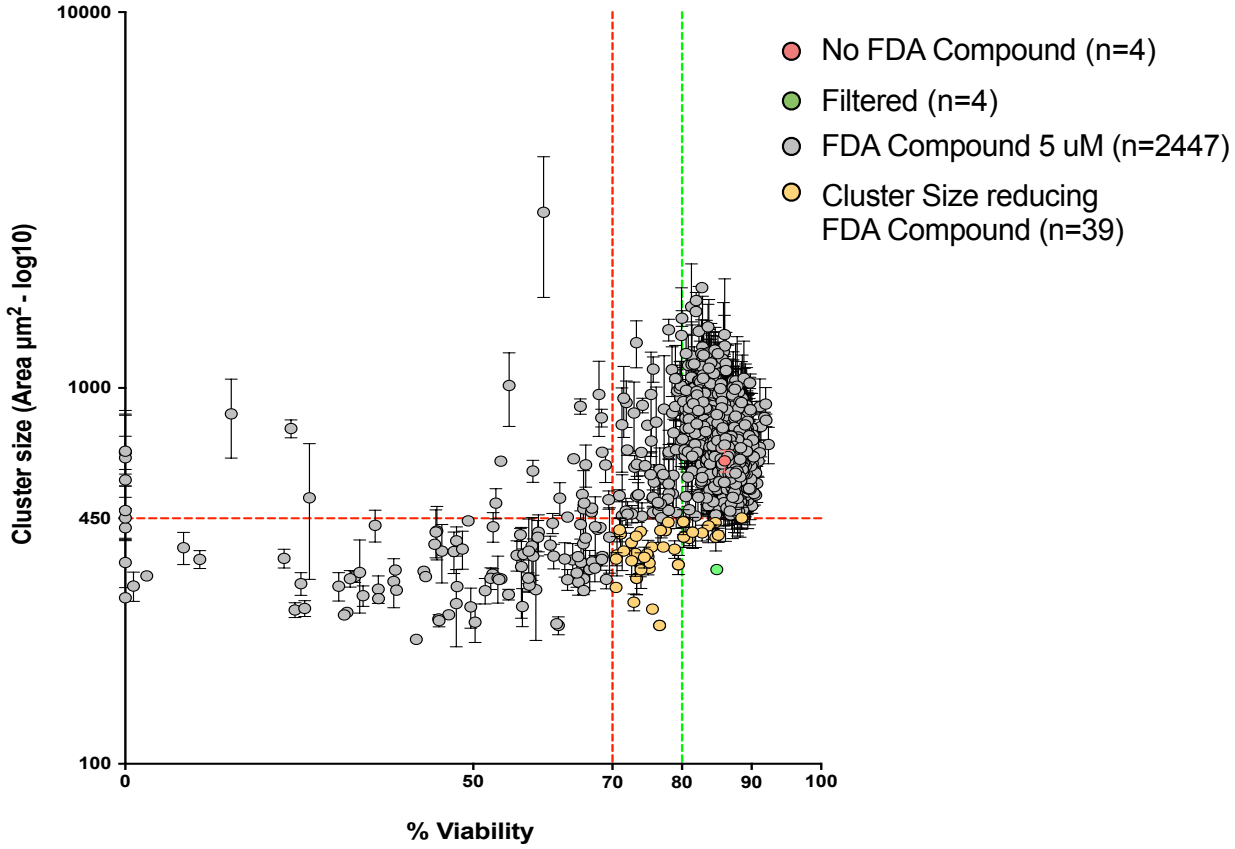
*Gkountela et al., **Cell**, 2019*

Clustering of CTCs induces a stem-like program



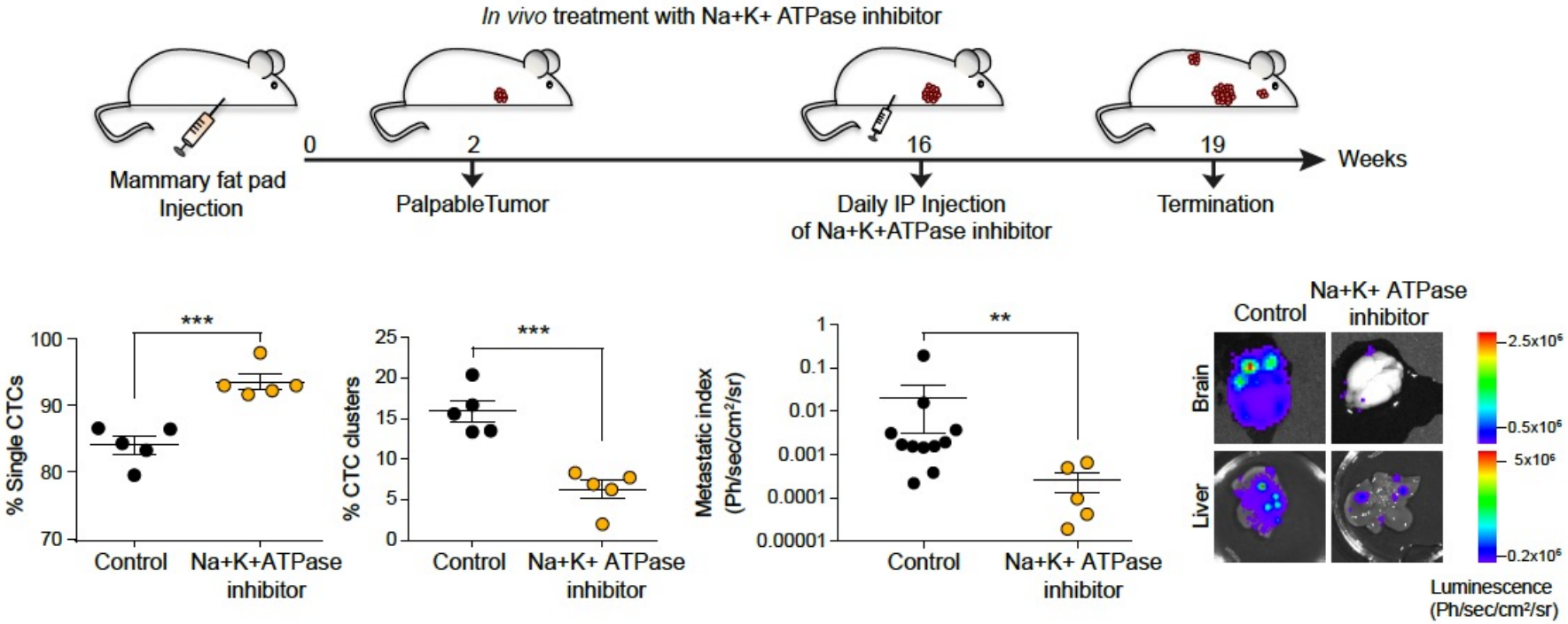
*Gkountela et al., **Cell**, 2019*

FDA-approved drug screen to disrupt CTC clusters

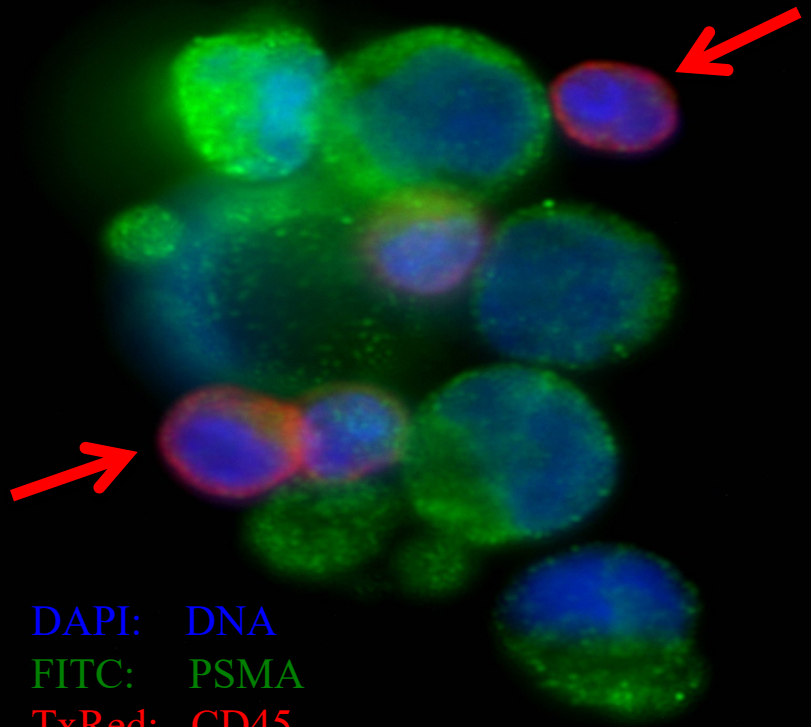


*Gkountela et al., **Cell**, 2019*

Treatment with cluster-targeting compounds suppresses metastasis in patient-derived xenografts



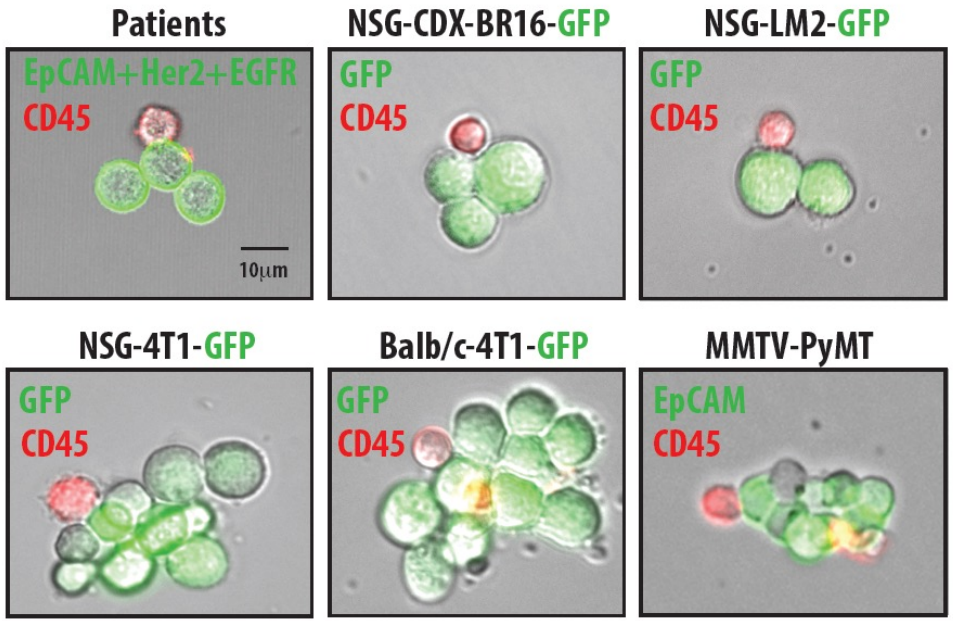
*Gkountela et al., **Cell**, 2019*

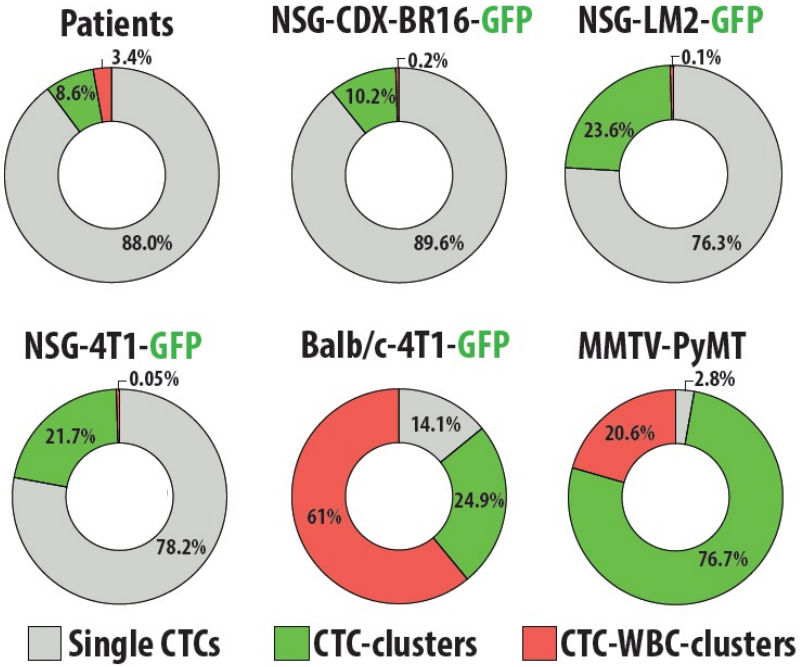


DAPI: DNA
FITC: PSMA
TxRed: CD45

Stott et al., **Science Transl Med**, 2010

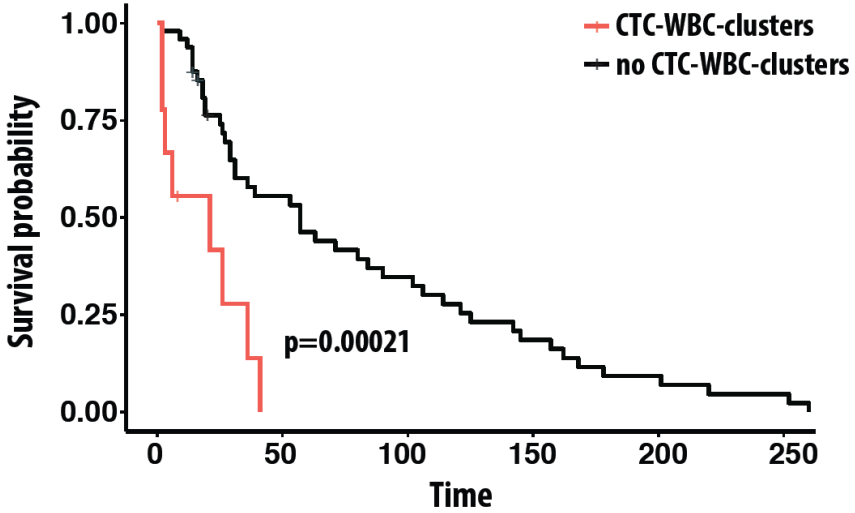
CTC-WBC clusters





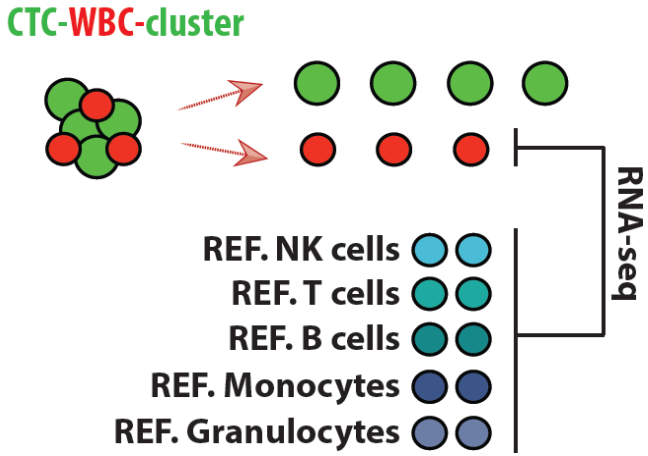
*Szczerba et al., **Nature**, 2019*

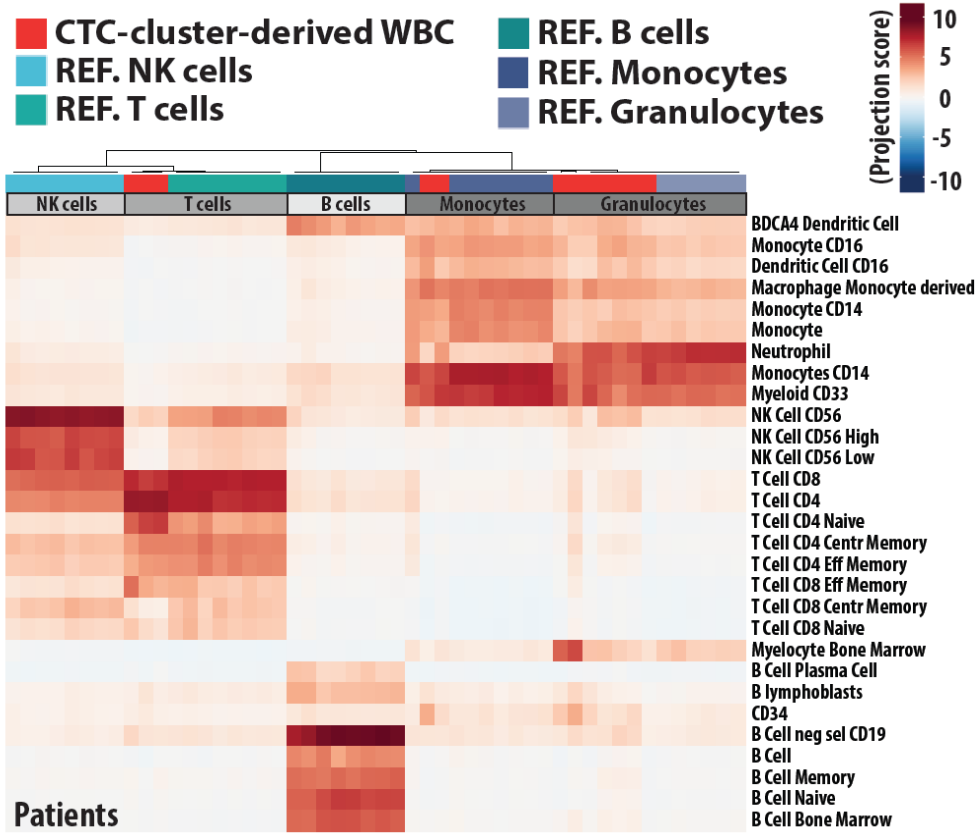
CTC-WBC clusters and short PFS



*Szczerba et al., **Nature**, 2019*

Single Cell Sequencing of CTC-WBC clusters





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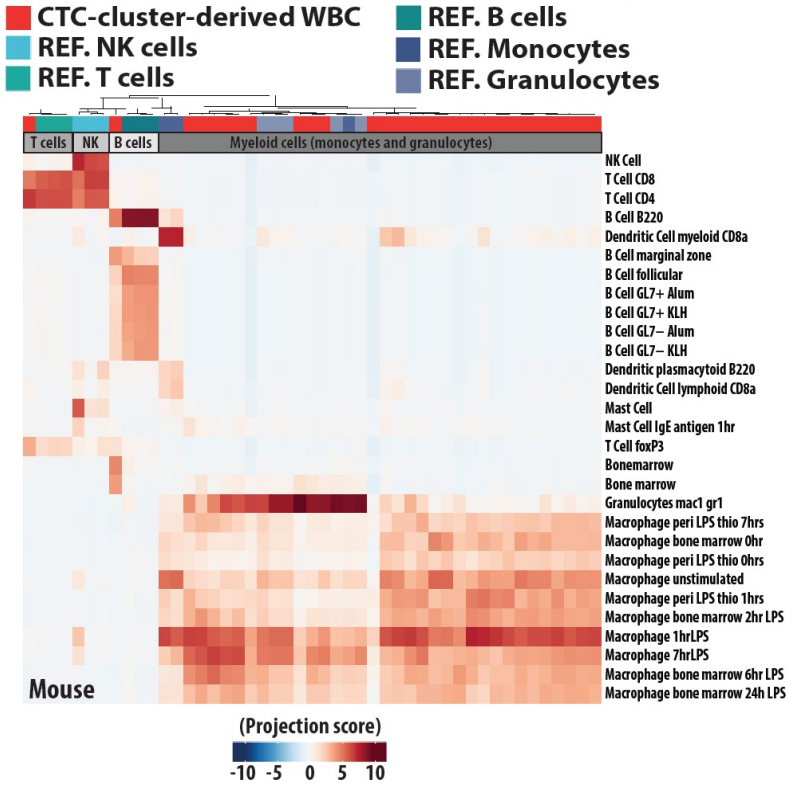

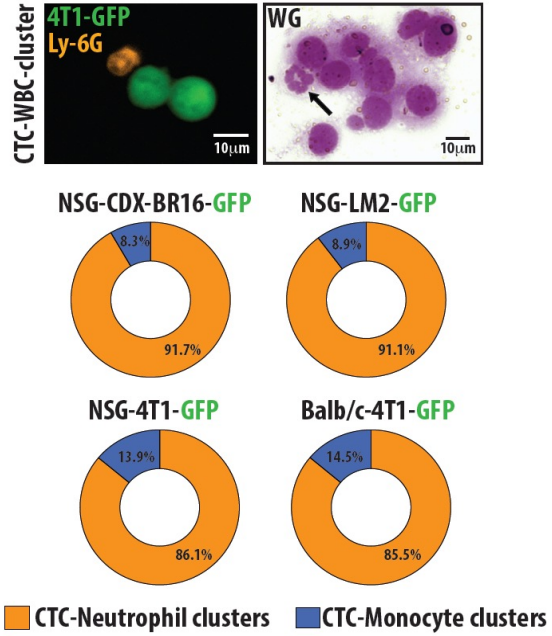

*Szczerba et al., **Nature**, 2019*

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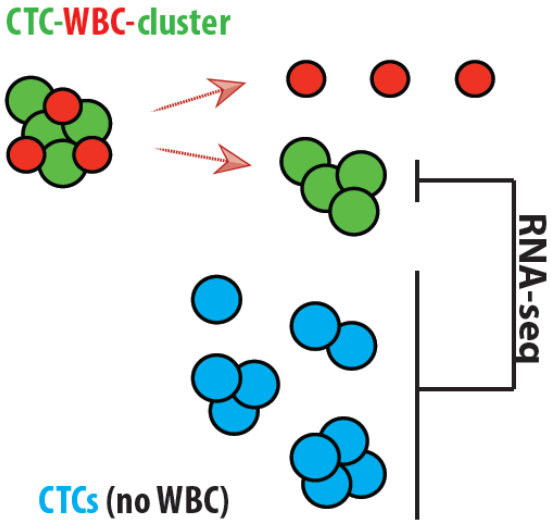
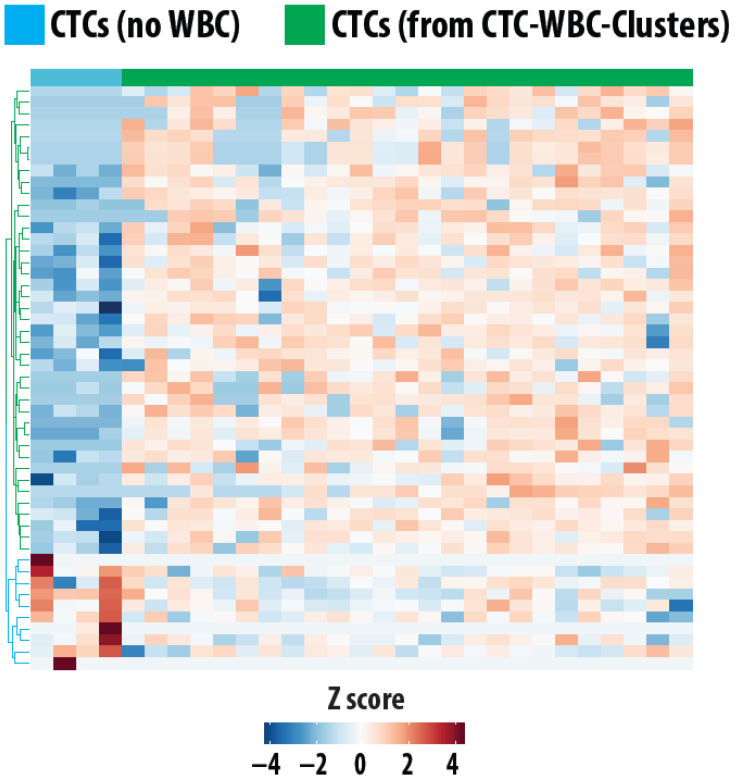
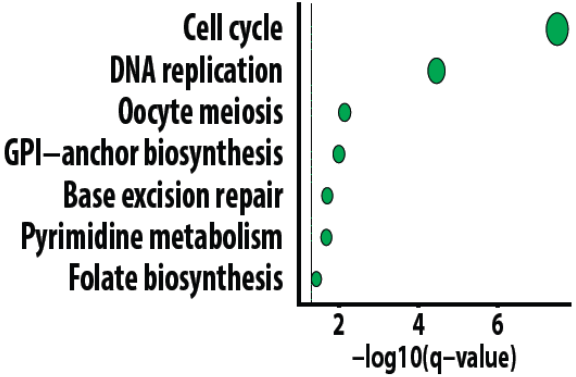
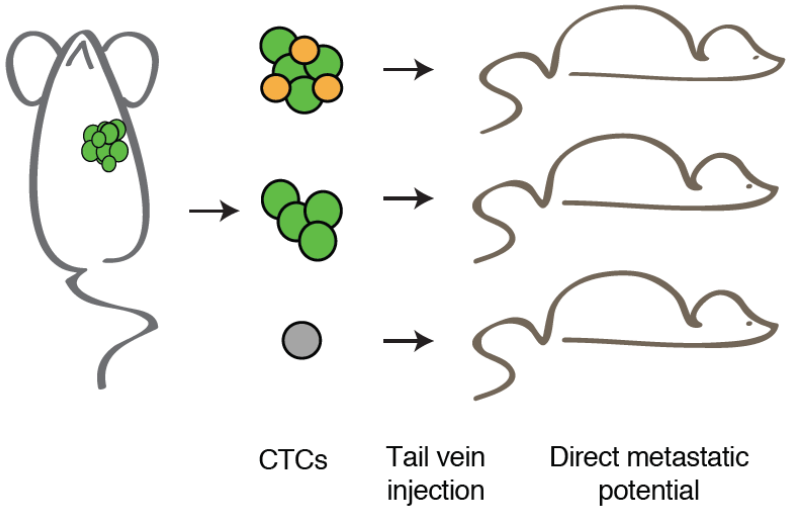


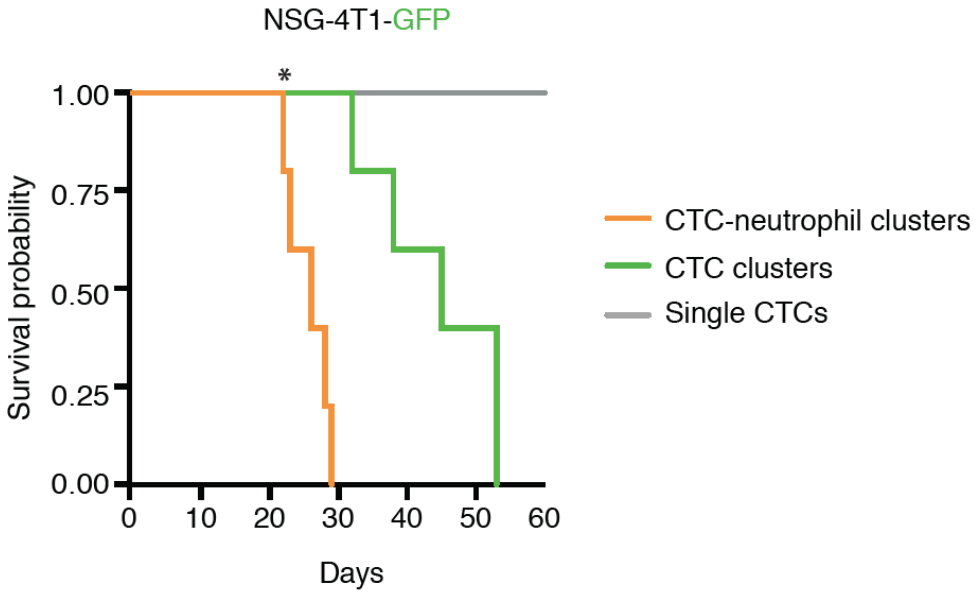




*Szczerba et al., **Nature**, 2019*

CTC-neutrophil clusters are the most metastasis-competent CTC subpopulation



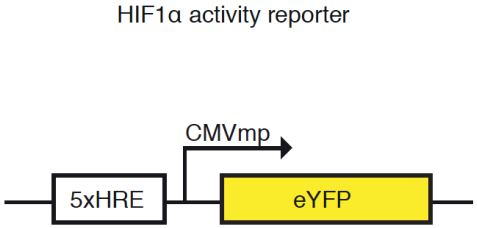


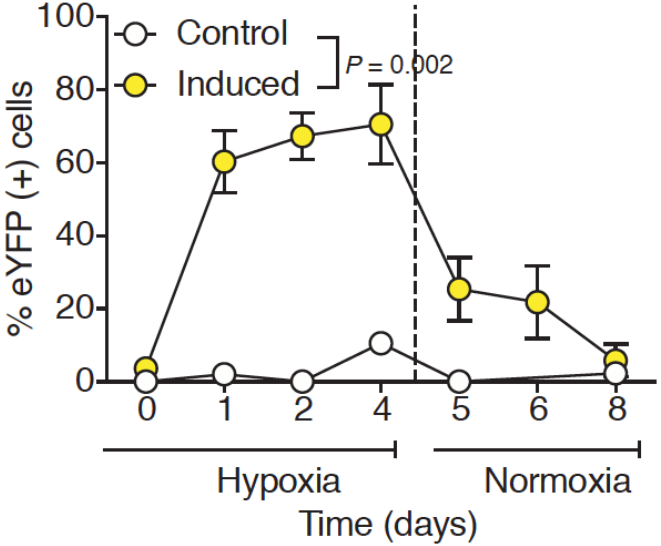
*Szczerba et al., **Nature**, 2019*

One step back: how do CTC clusters form?

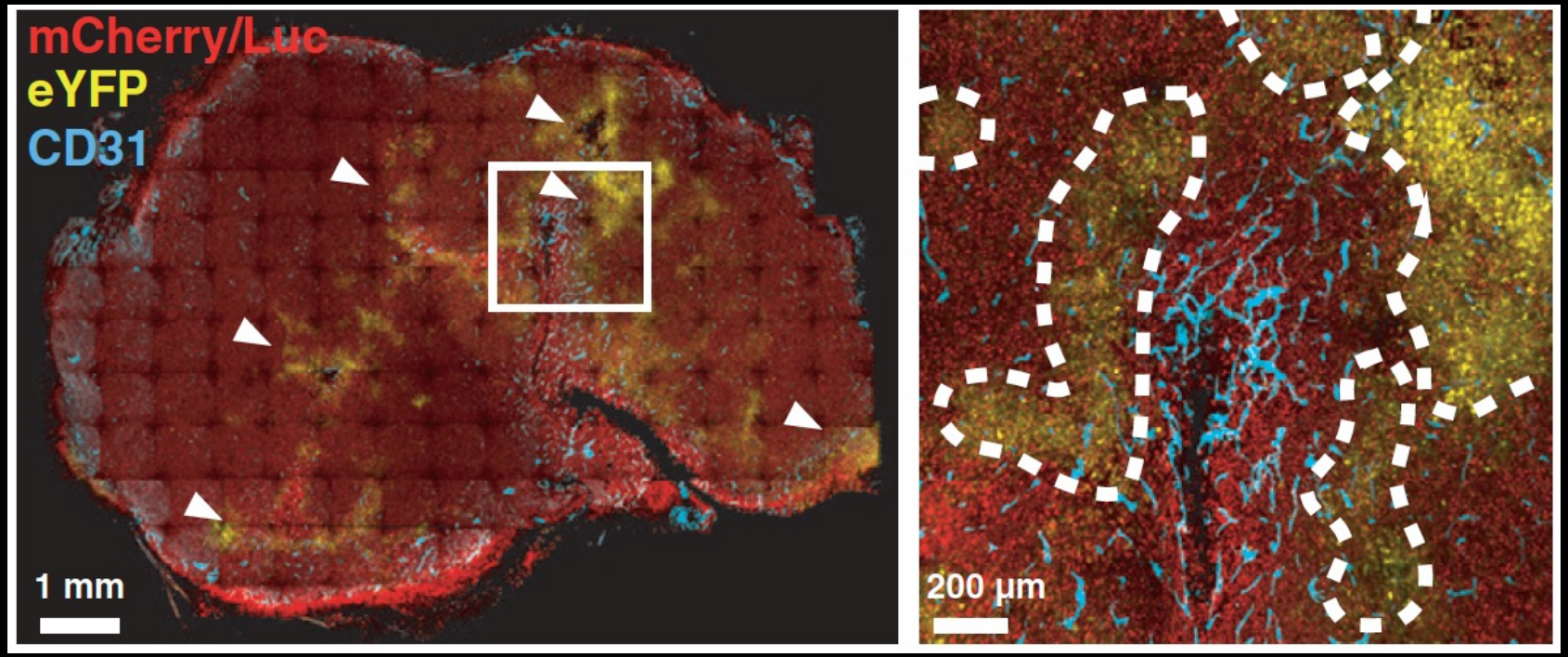
Intra-tumor Hypoxia: a link with CTC clusters formation?

Hypoxia reporter





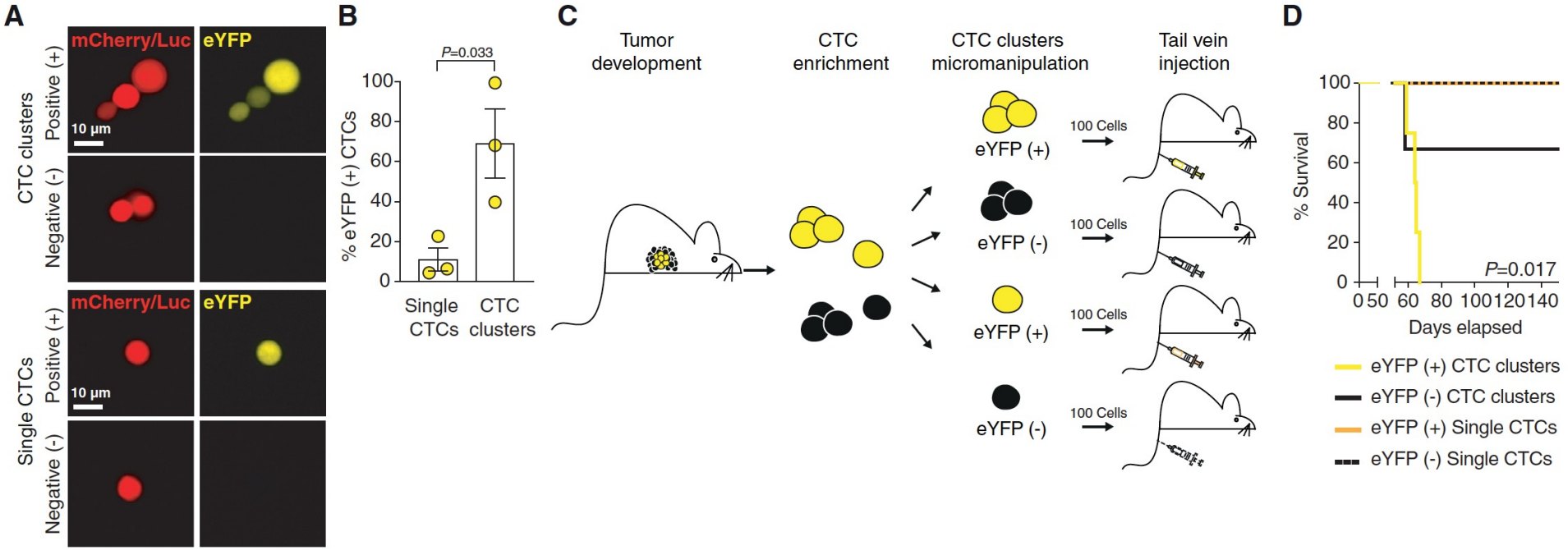
*Donato et al., **Cell Rep**, 2020*



Yellow: hypoxia

*Donato et al., **Cell Rep**, 2020*

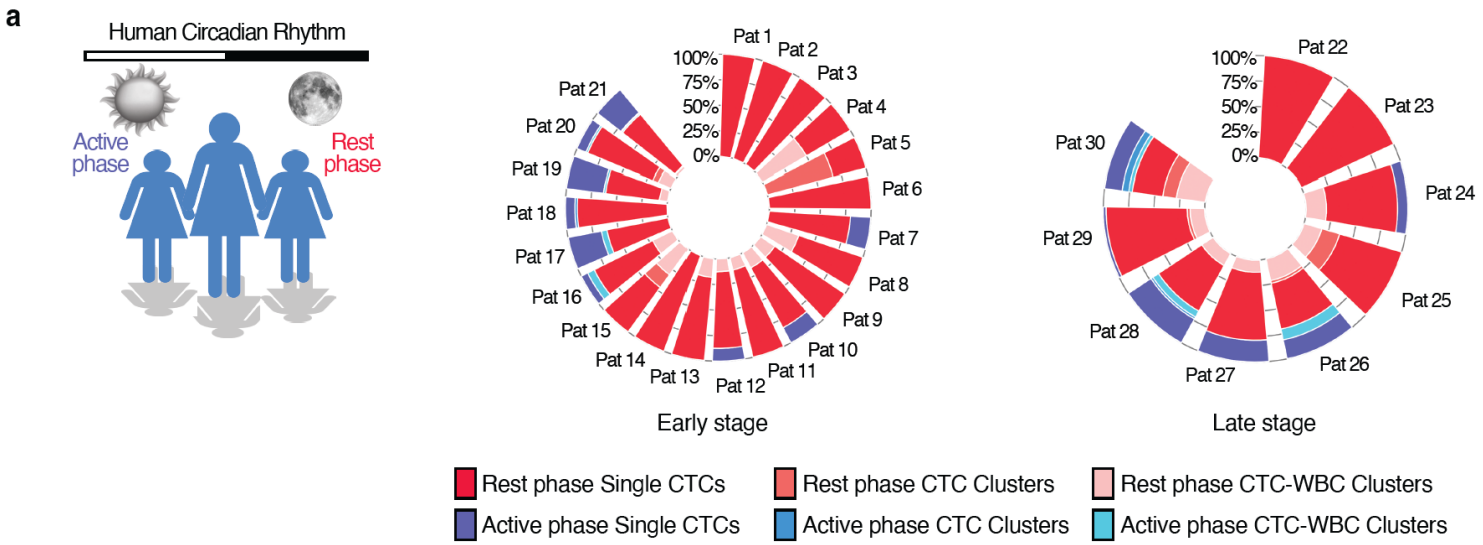
CTC clusters are Hypoxic, single CTCs are normoxic



*Donato et al., **Cell Rep**, 2020*

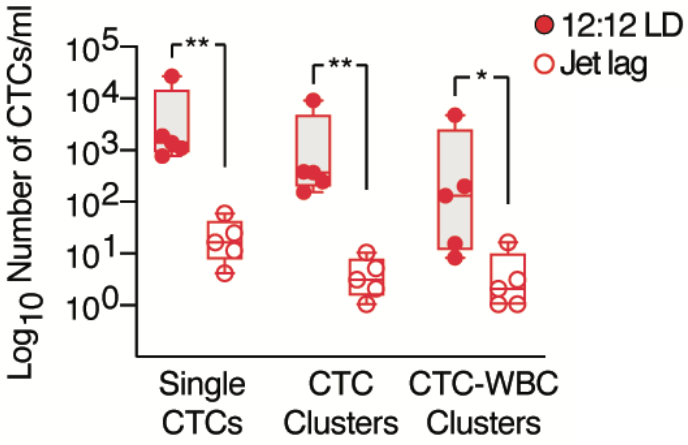
Two steps back: ***when*** *are CTCs formed?*

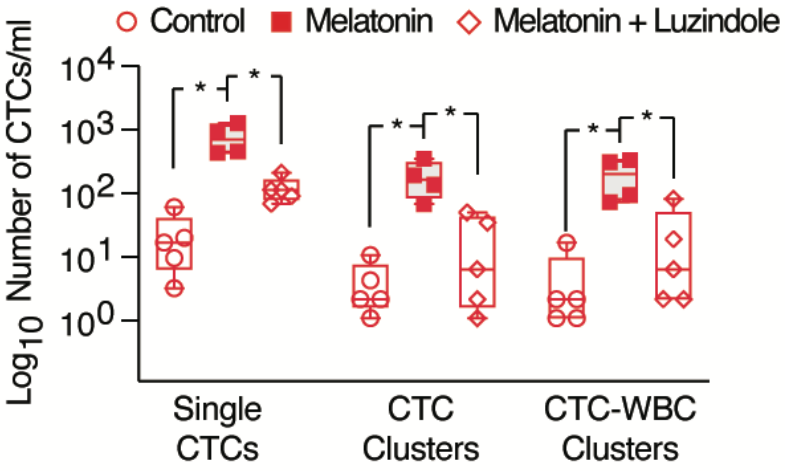
Most CTCs are produced during sleep



*Diamantopoulou et al., **Nature**, 2022*

Most CTCs are produced during sleep





*Diamantopoulou et al., **Nature**, 2022*

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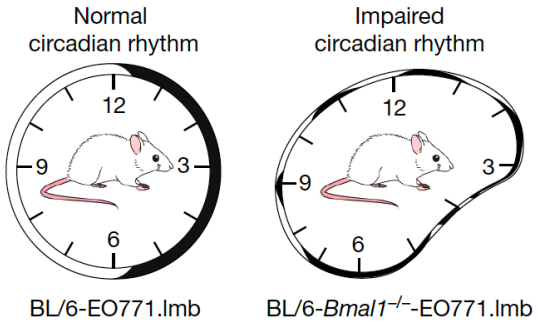
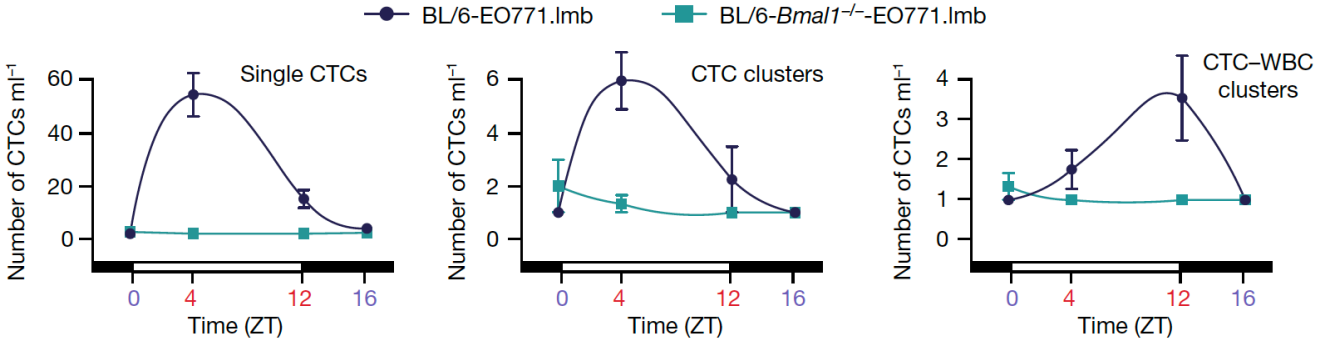
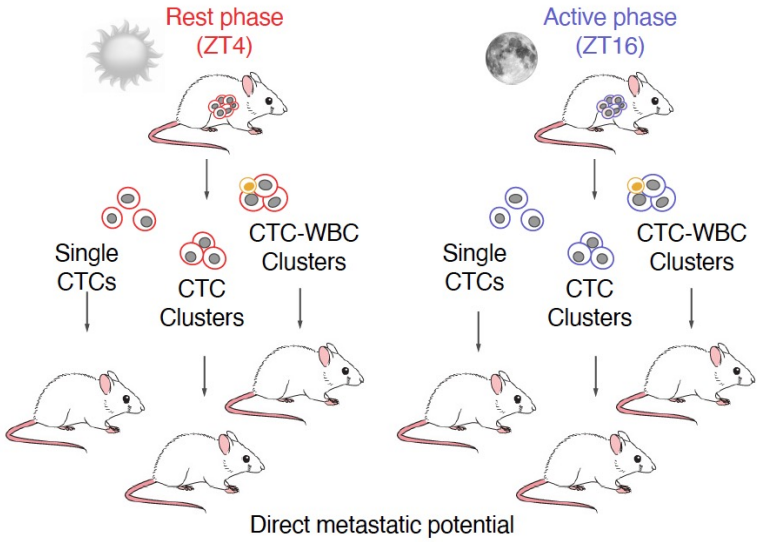
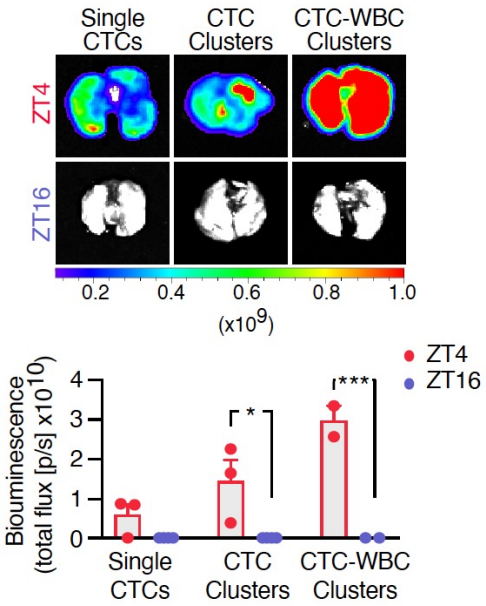




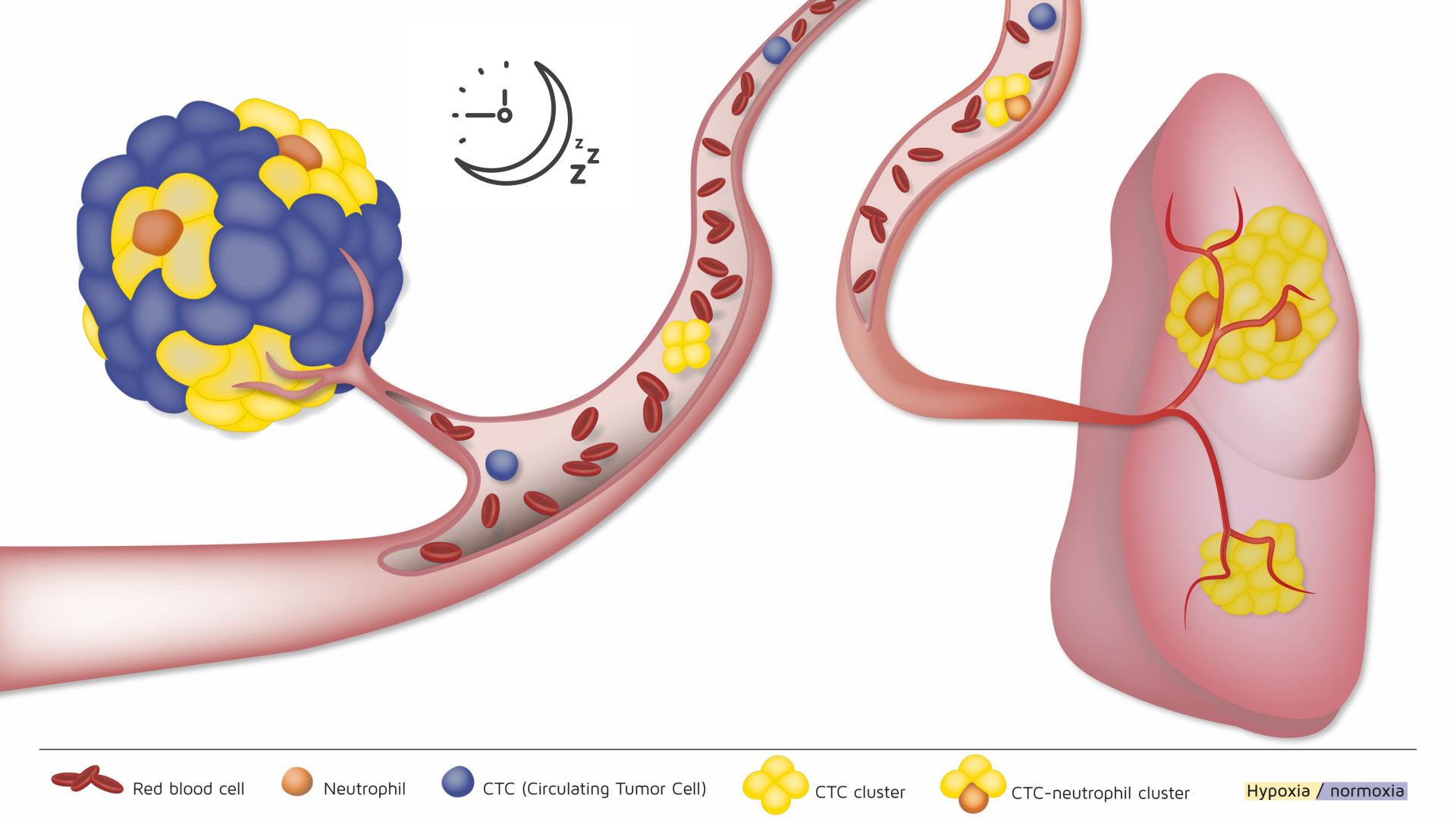
Diamantopoulou et al., ***Nature***, *2022*

Metastases are formed during sleep



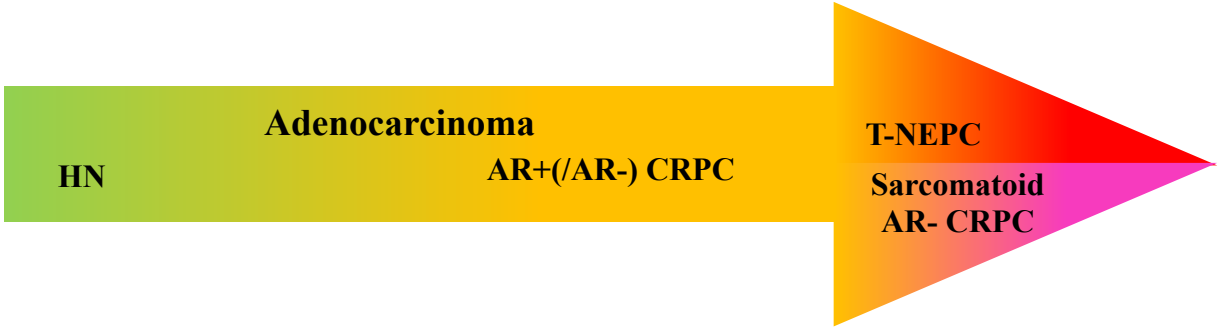


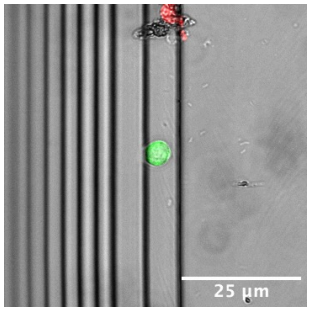
*Diamantopoulou et al., **Nature**, 2022*


Red blood cell
Neutrophil
CTC (Circulating Tumor Cell)
CTC cluster
CTC-neutrophil cluster
Hypoxia / normoxia

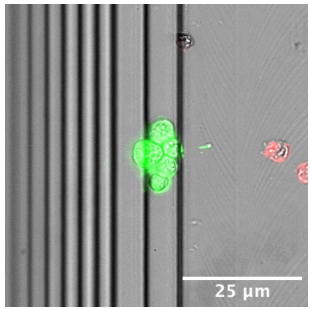
Insights into prostate CTCs

Insights into prostate CTCs

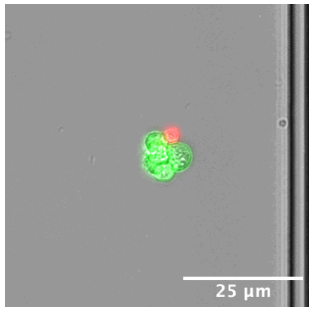




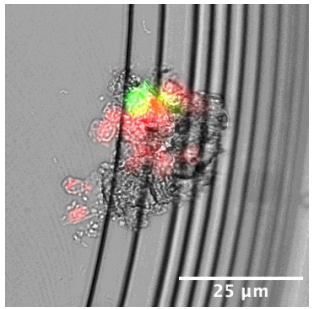
Single CTC



Homotypic CTC Cluster



Heterotypic CTC-WBC Cluster



Heterotypic Mixed CTC Cluster

PSMA
EpCAM
HER2
EGFR
CD45

Confidential, DO NOT POST

Take home

- ✓ CTC clusters are precursors of metastasis in breast cancer
- ✓ CTC clusters display stem cell-like properties
- ✓ We identified drugs that can dissociate CTC clusters and suppress metastasis in preclinical models of breast cancer: now testing in patients
- ✓ Neutrophils may associate to CTCs to maintain CTC proliferation in the bloodstream and increase metastasis seeding
- ✓ Low oxygen in tumors leads to cell-cell junction upregulation and intravasation of clustered CTCs (with high metastatic ability)
- ✓ CTC intravasation and metastatic ability is highest during sleep