Convergent Evolution and the Origins of Lethal Cancer

Kenneth J. Pienta, MD
Donald S. Coffey Professor of Urology
Professor of Urology, Oncology, Pharmacology and Molecular Sciences, and Chemical and Biomolecular Engineering, Johns Hopkins University
Director, Brady Urologic Research Institute

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BRADY 100 YEARS
1915 - 2015

Cancer is an ongoing health *crisis*.

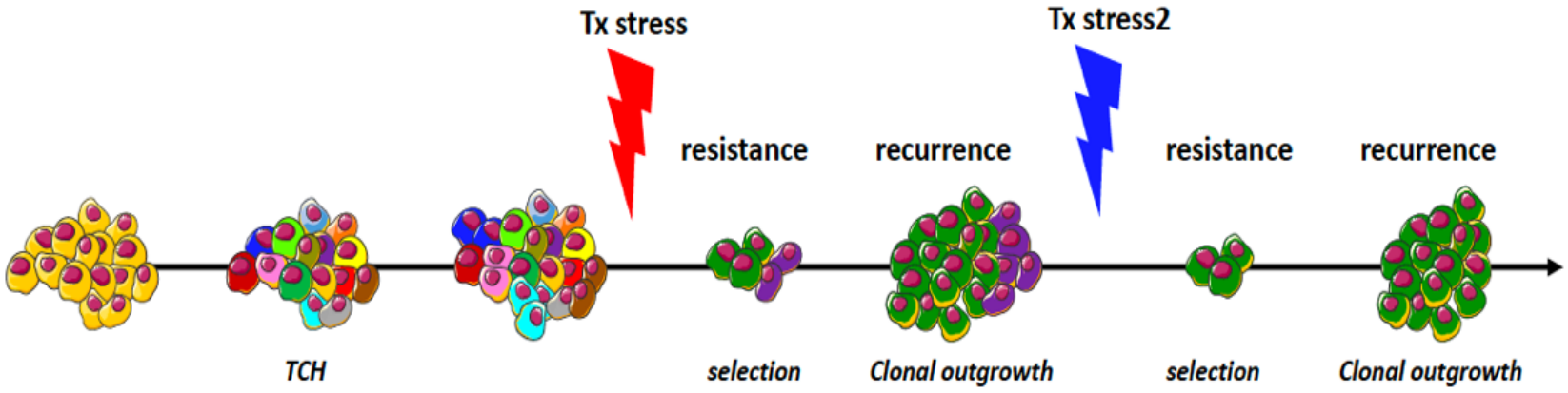
Cancer kills 10 million people a year globally.

In the United States, 600,000 people are dying every year from cancer.

1 person is dying every minute.

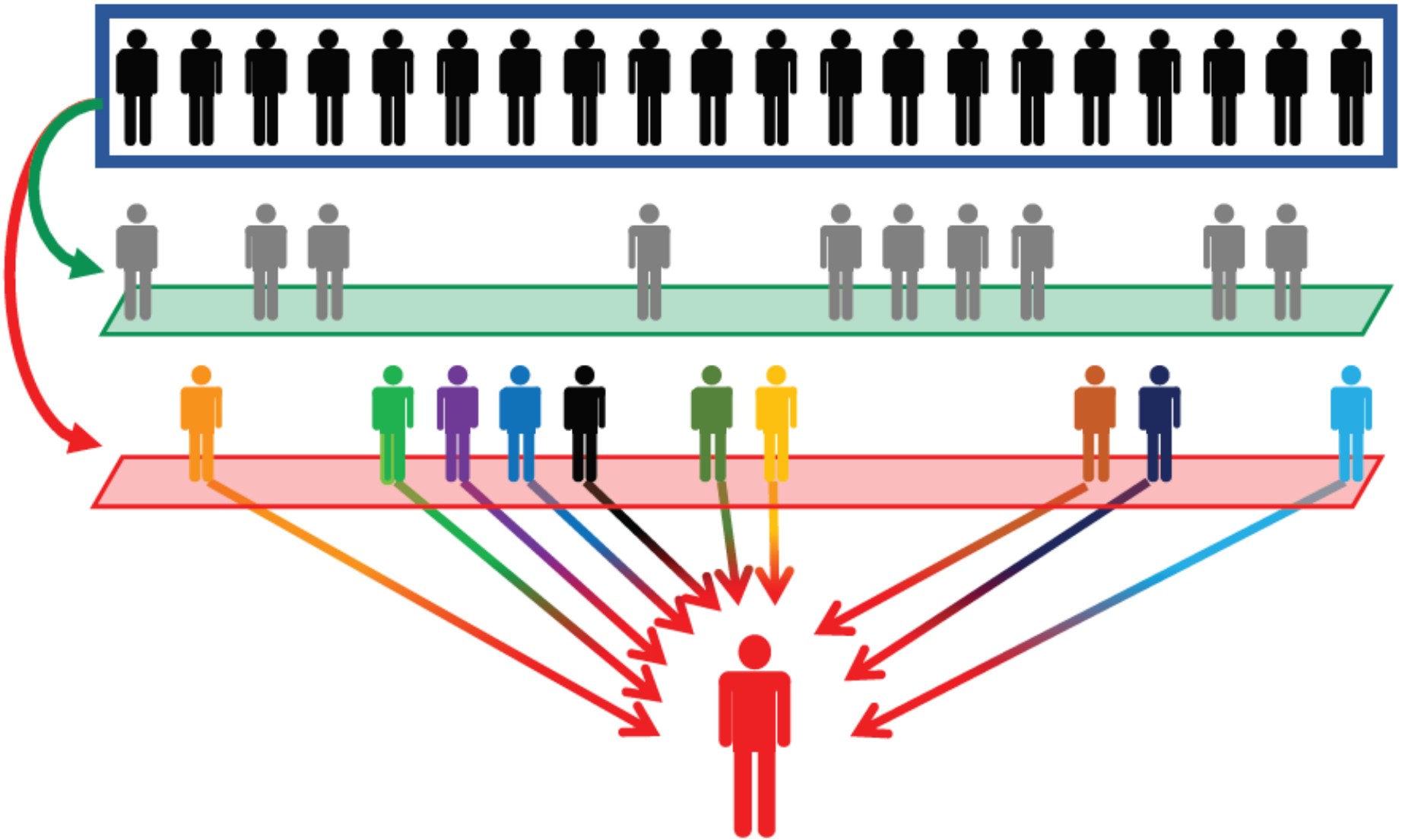
Cancer kills people for two reasons:

- **It spreads to all parts of the body (metastasis).**
- **It is resistant to all known forms of systemic treatment.**
  - **Cancer is only routinely cured if it can be cut out or killed with focused radiation.**
    - Traditionally, this has been explained by the thought that within the billions of cancer cells in a tumor, resistance to therapies evolves by random chance that endows at least one cancer cell with resistance to any particular therapy.
    - This explanation relies on chance since lethal cancer demonstrates resistance to therapeutic agents that it has previously not been exposed to.



Metastatic Cancer is ultimately resistant to virtually all systemic therapies.

- From an evolutionary ecology perspective – these two processes, both requiring resiliency and the same co-adaptations, are likely linked.
  - Cancer arises independently and is lethal in 10 million people per year
  - We believe that the **lethality** resulting from metastasis and resistance an example of **convergent evolution.**



20 million
cancer diagnoses

10 million
cancer patients cured
with local therapy

10 million
cancer patients progress
with metastatic and
therapy resistant disease

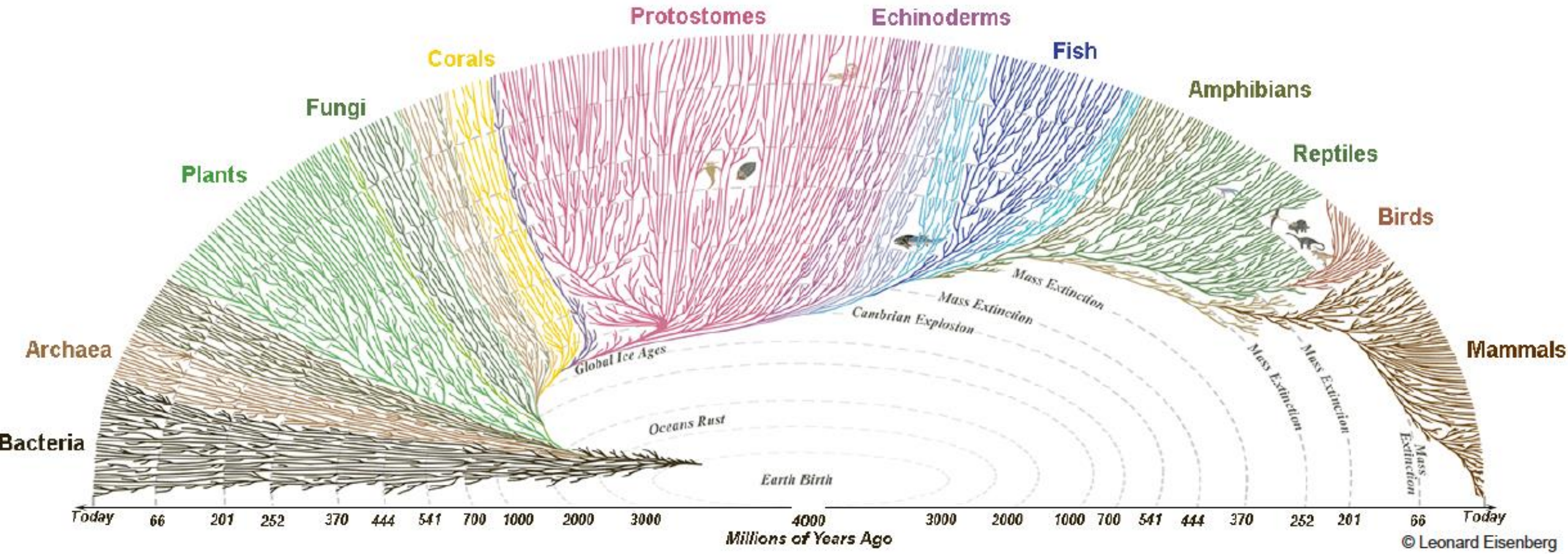
**1 convergent
lethal cancer
phenotype**

Mol Cancer Res. 2020 Jun;18(6):801-810.

Convergent evolution

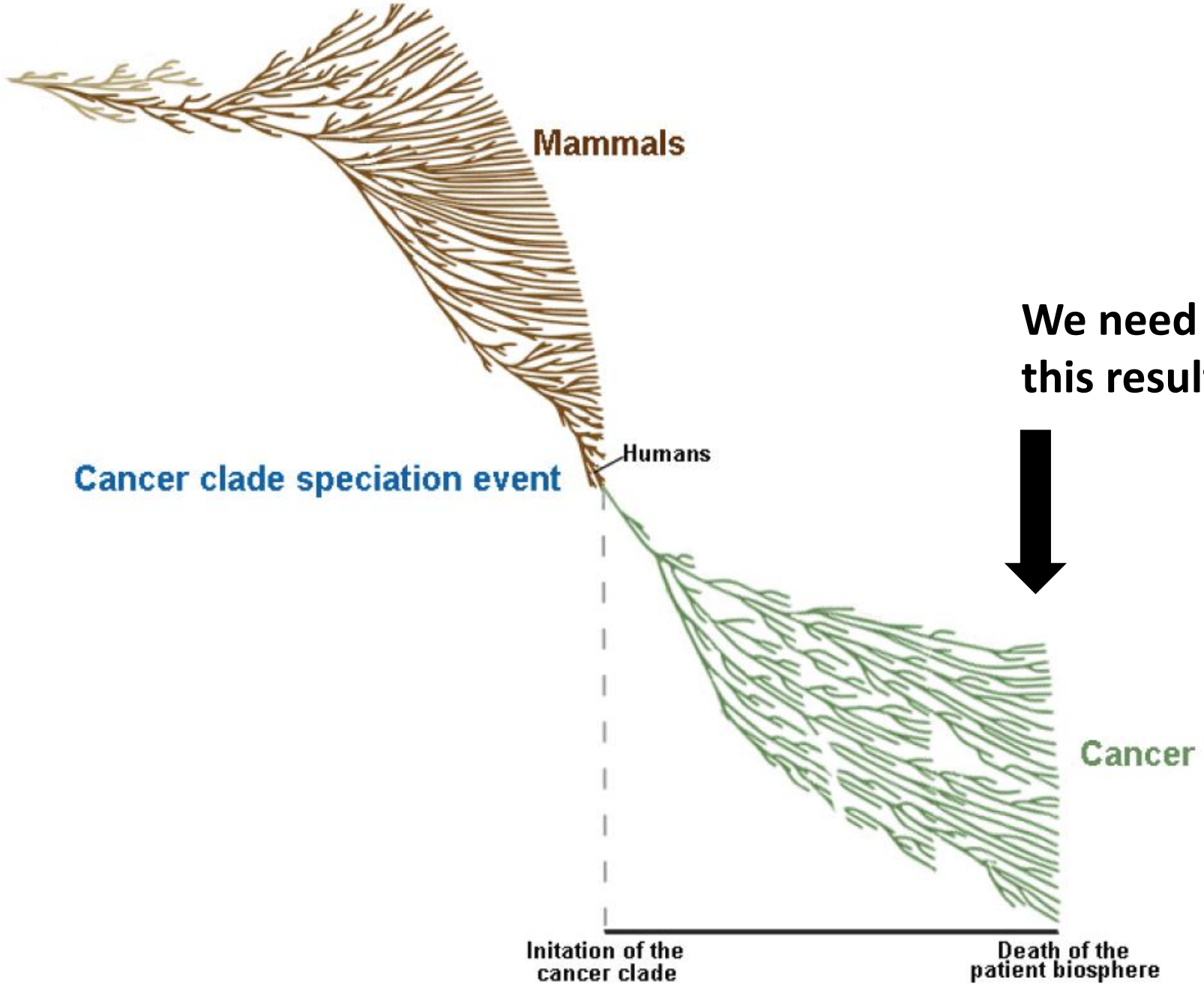
- **Convergent evolution** is the independent evolution of similar features across species of different periods or epochs in time. Convergent evolution creates **analogous structures** that have similar form or function but were not present in the last common ancestor of those groups.
  - Wings
  - Hooves
  - Teeth
  - Eyes

Evolutionary clades



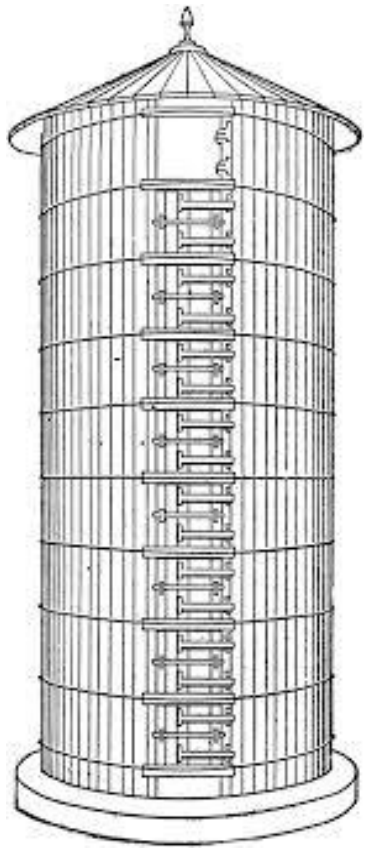
A clade is a monophyletic group derived from a common ancestor and including all its lineal descendants.

Mol Cancer Res. 2020 Jun;18(6):801-810.

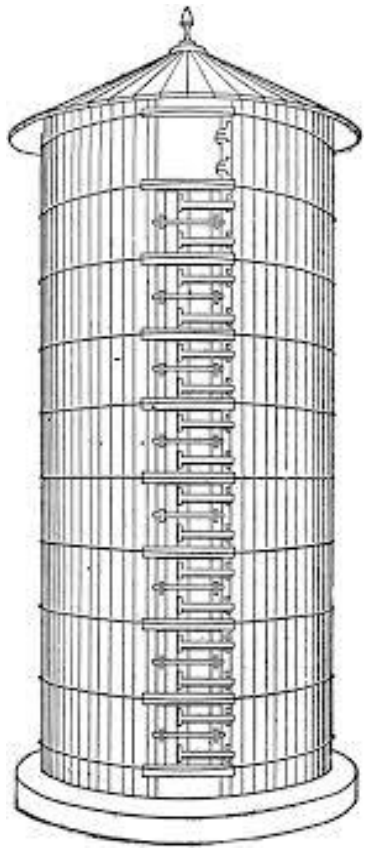


We need to understand why this results in lethality

Classically, metastasis and resistance are considered two distinct processes, attributed to tumor heterogeneity but studied by different groups of scientists.

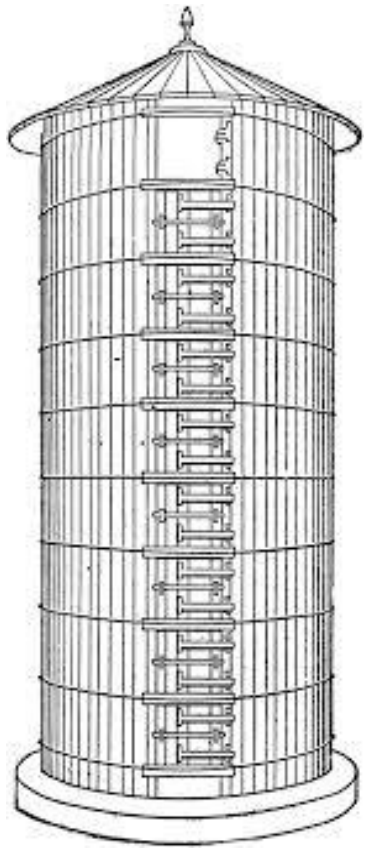


METASTASIS



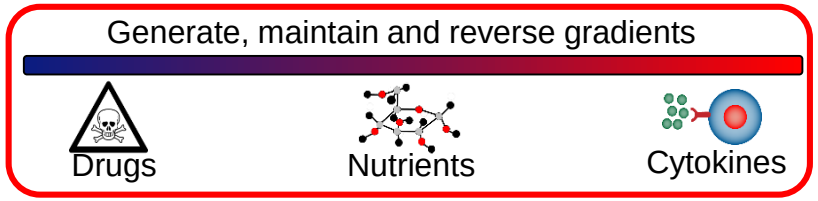
RESISTANCE

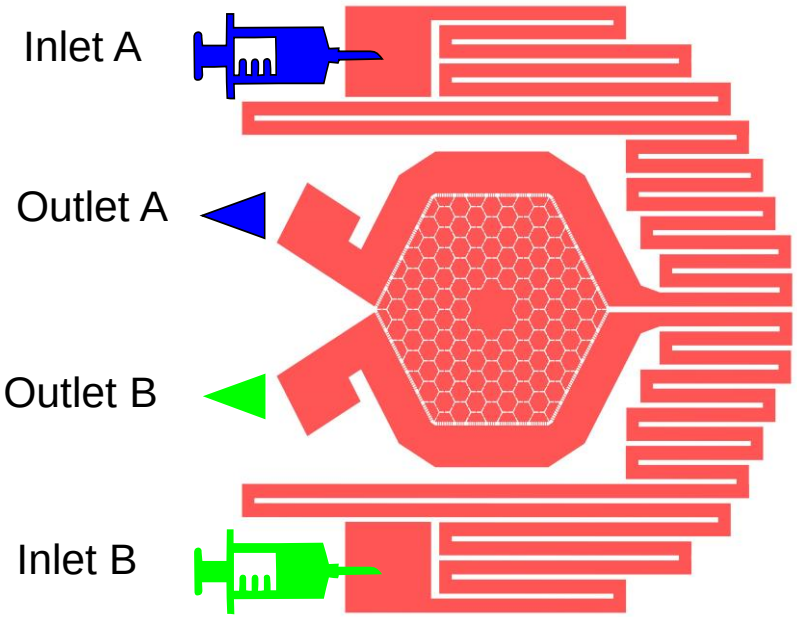
Can we explain metastasis and resistance within a single silo of study?



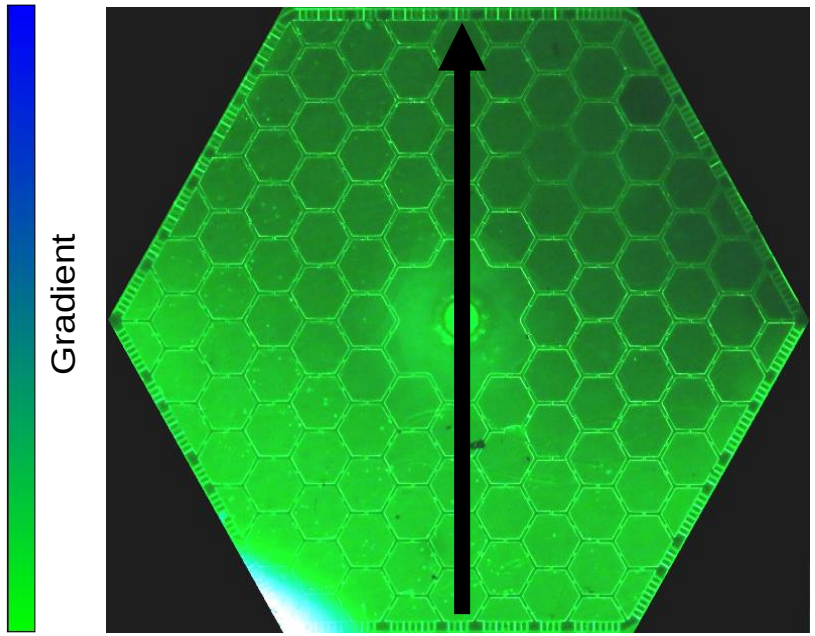
METASTASIS + RESISTANCE

Modeling of metastasis and resistance by creating the ‘cancer swamp’

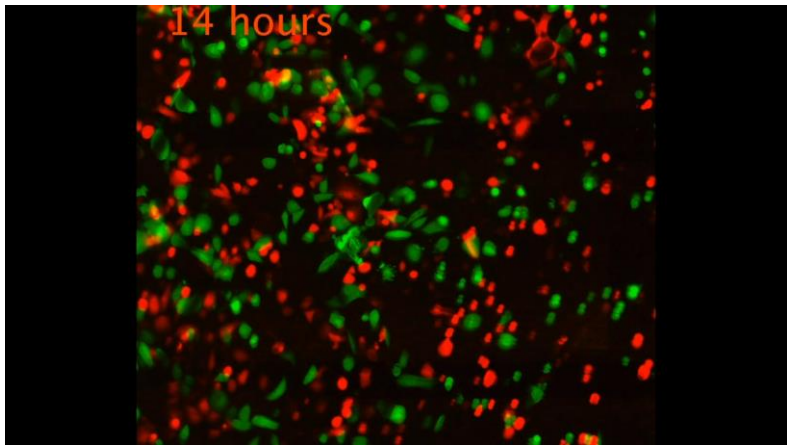




Microfluidics Array



Fluorescein Gradient



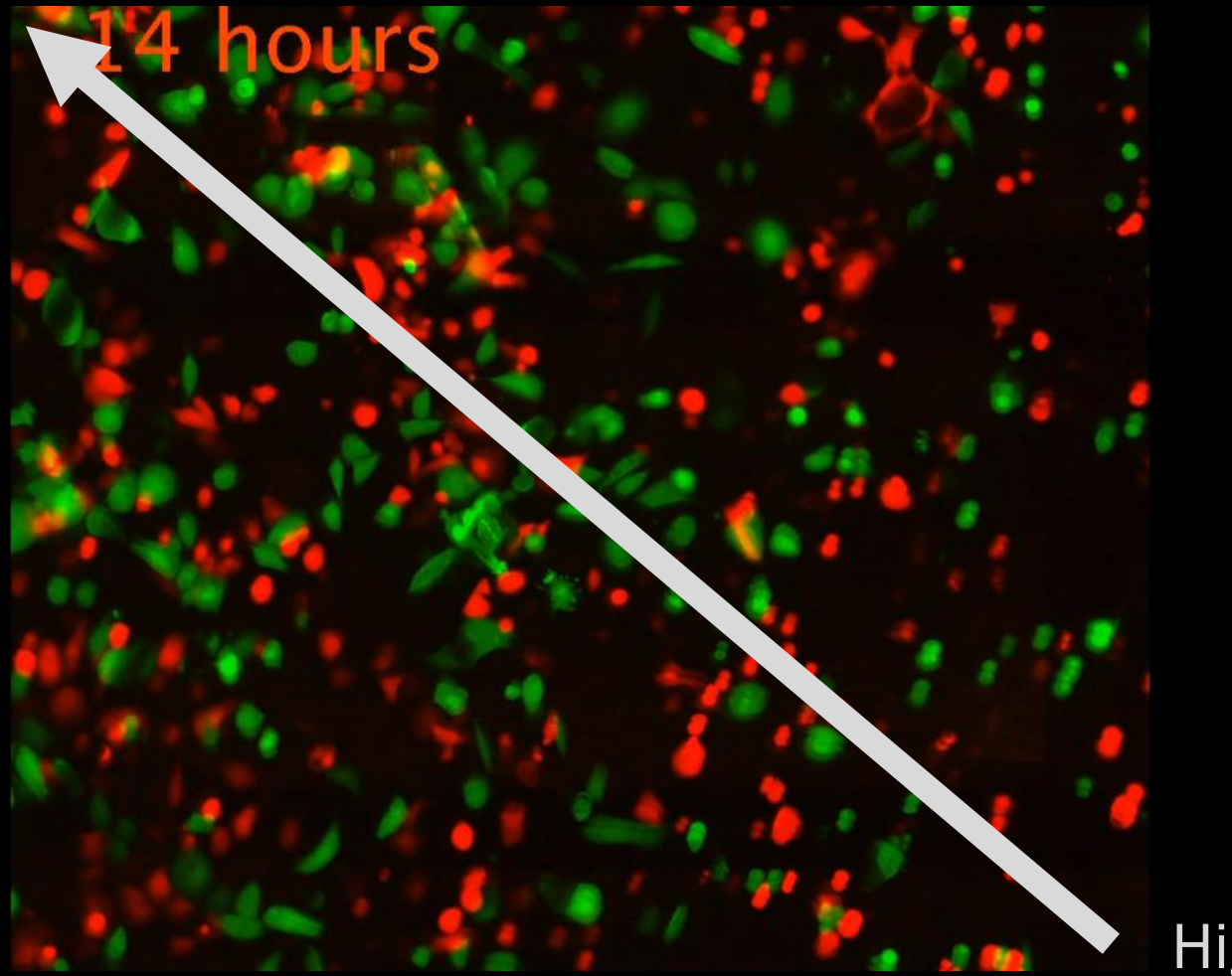
Automated tracking

Lab Chip. 2020 Jul 14;20(14):2453-2464.

Converg Sci Phys Oncol. 2017 Dec;3(4):045001.

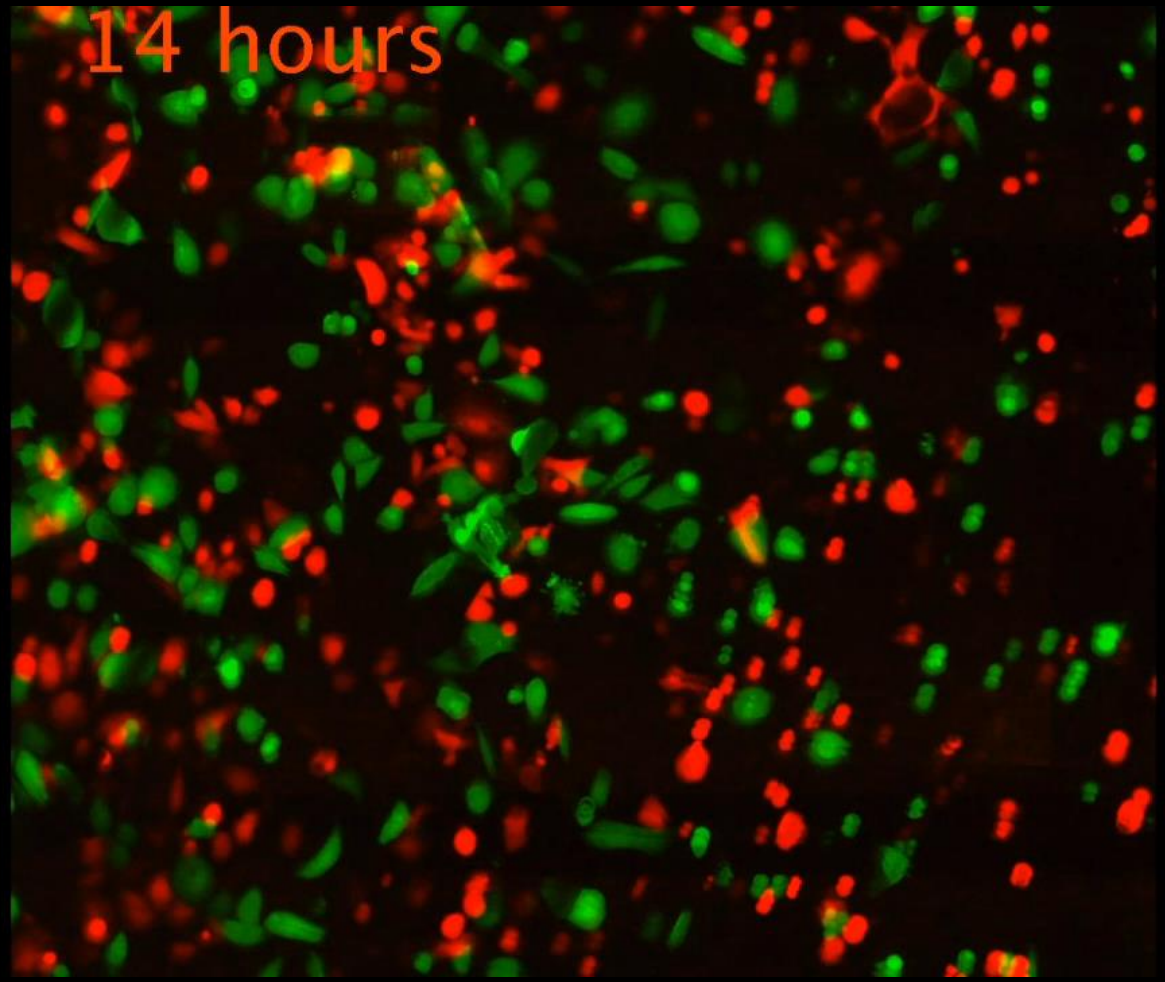
PC3 prostate cancer cells in lethal chemotherapy

Low docetaxel

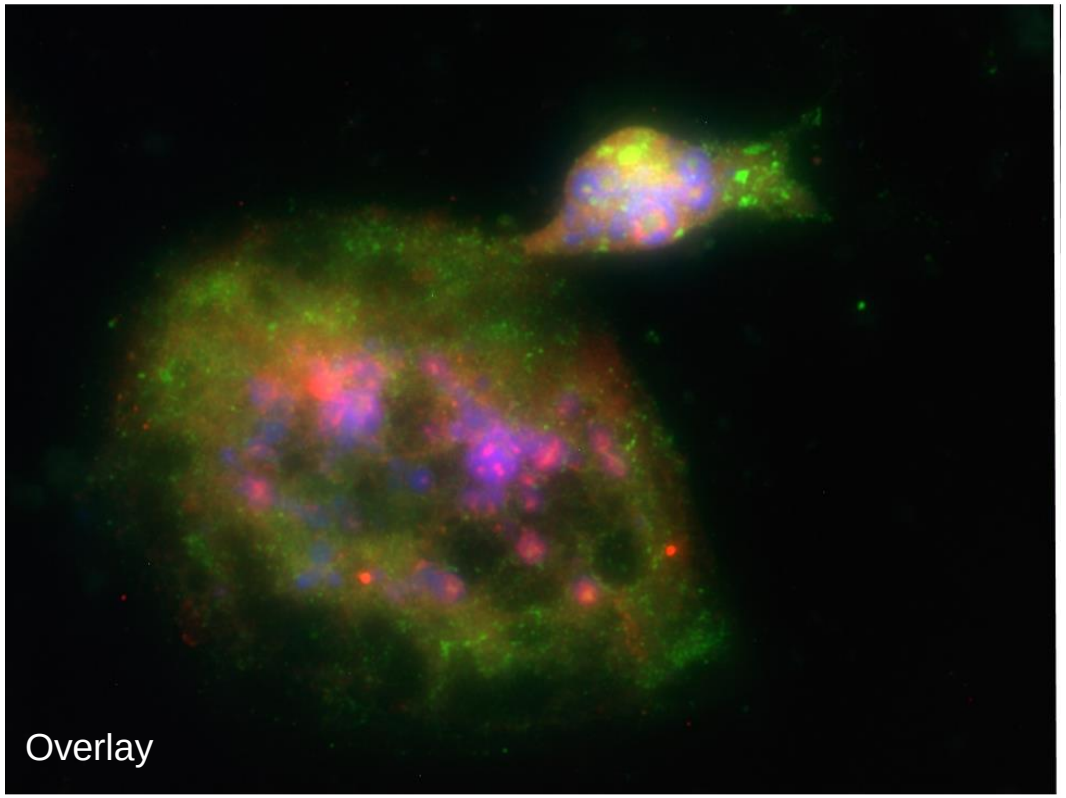


High docetaxel

PC3 prostate cancer cells in lethal chemotherapy



Polyaneuploid cells can asymmetrically divide and generate $2N^{+}$ cells (“bloom”)



A. Aneuploid
B. Polyploid

1. Highly motile
2. Highly resistant
3. Seed recurrence

40X

PACCs first described in 1858
Rudolf Virchow (Father of Modern Pathology)

1858: <u>Cellular Pathology as based upon Physiological and Pathological Histology</u>

Fig. 142

Various, polymorphous cancer-cells, some of them in a state of fatty degeneration, two with multiplication of nuclei. 300 diameters.

FIG. 142.

Prostate. 2019 Sep;79(13):1489-1497.

Multinucleated polyploid cells have been reported in the literature

The formation of giant multinucleated polyploid cells after therapeutic intervention has been well described

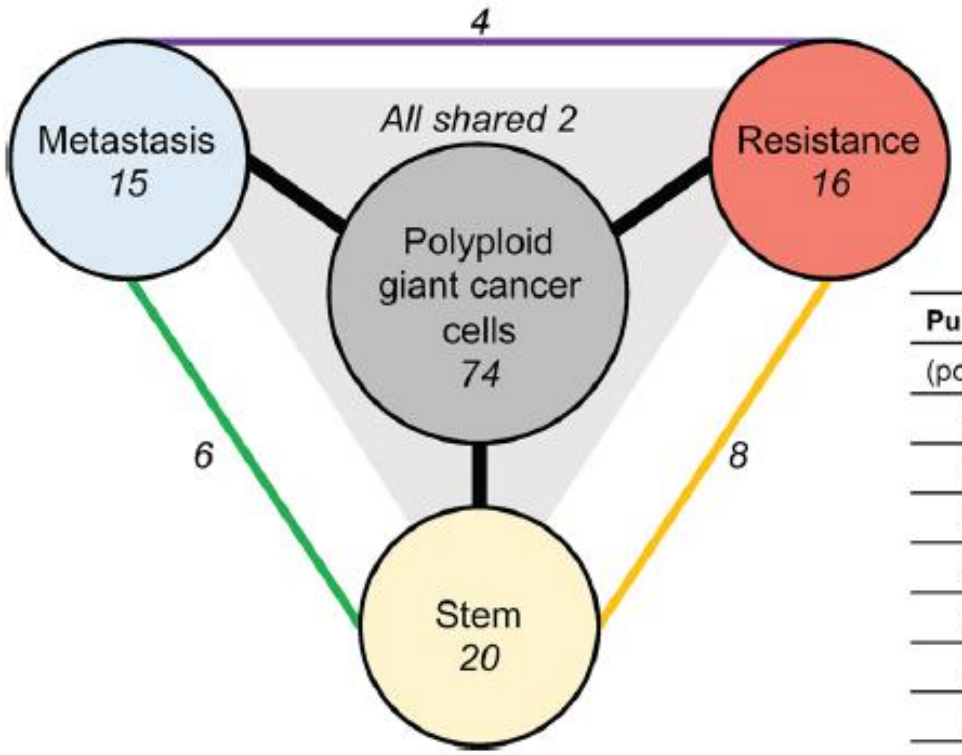
- Chemotherapy
- Radiotherapy
- Tumor microenvironment

TALK: Jinsong Liu: 9:15 am, October 16th

It has been assumed by the majority of the cancer community that these giant polyploid cells do not survive and die due to mitotic catastrophe subsequent to multipolar cell division or *simply senesce.*

Evol Appl. 2020 Feb 22;13(7):1626-1634

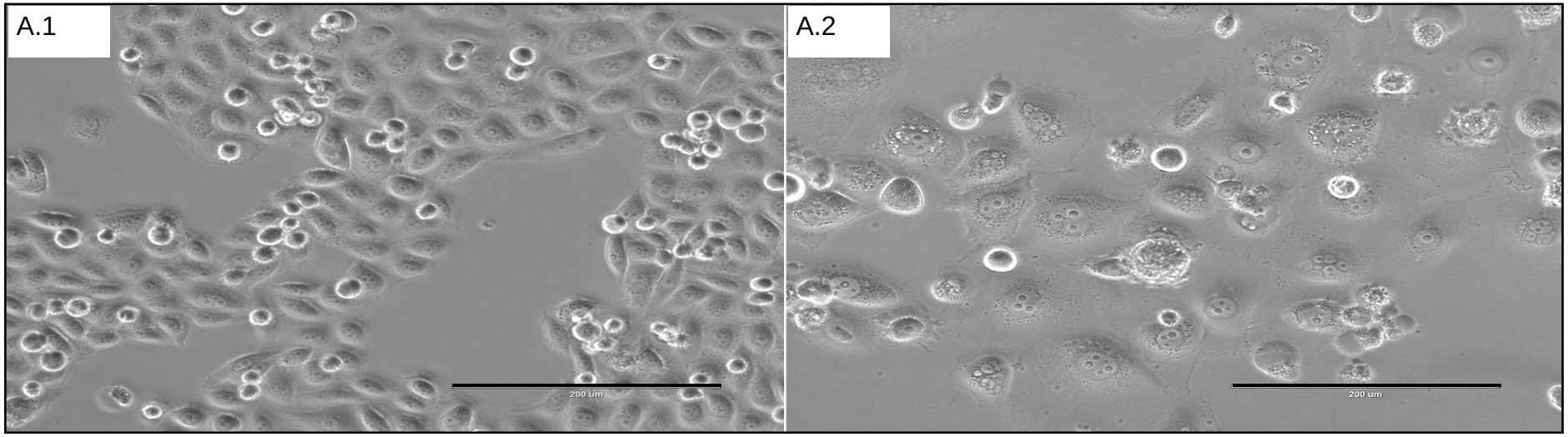
More silo’ed research

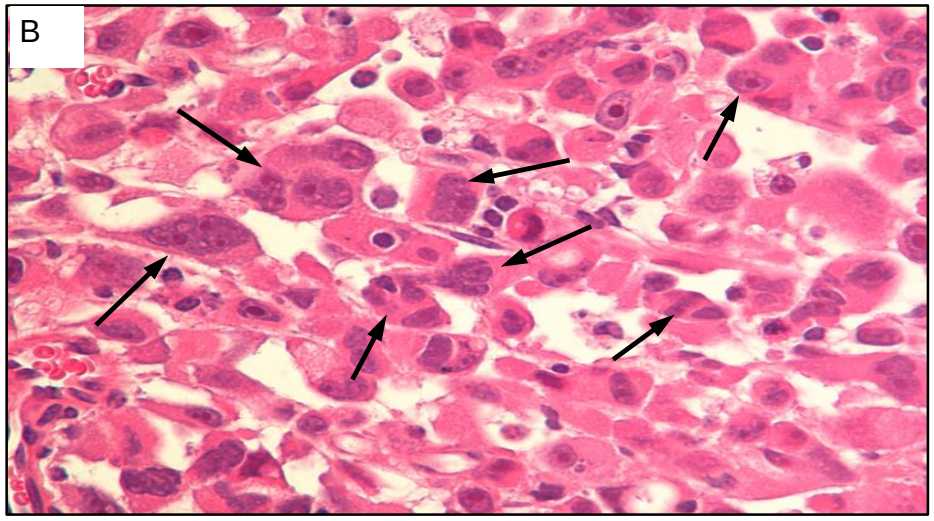


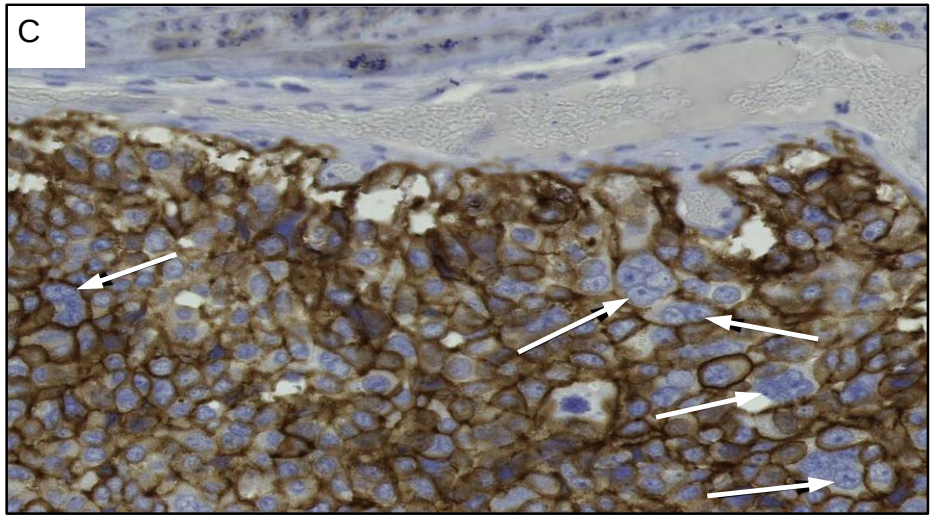
PubMed query	entries
(polyploid giant cancer cells)	74
AND (metastasis)	15
AND (stem)	20
AND (resistance)	16
AND (metastasis) AND (resistance)	4
AND (metastasis) AND (stem)	6
AND (resistance) AND (stem)	8
AND (metastasis) AND (resistance) AND (stem)	2

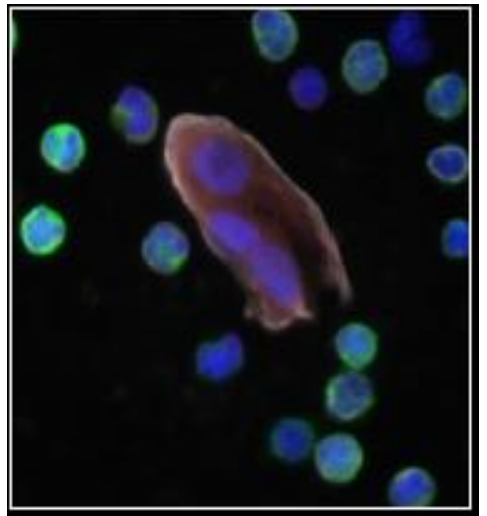
Prostate. 2019 Sep;79(13):1489-1497.

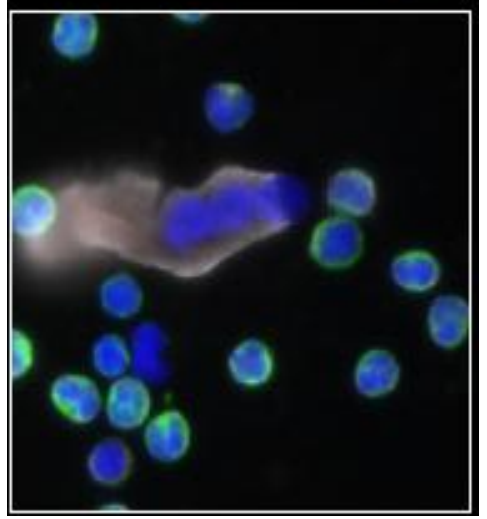
Polyaneuploid cancer cells (PACCs) are central actuators of tumorigenesis, metastasis, and therapeutic resistance



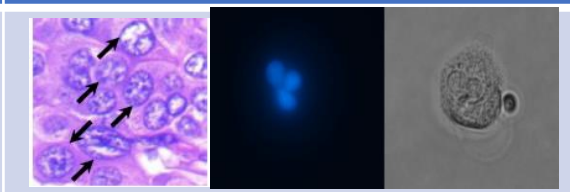
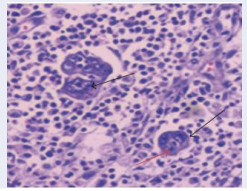
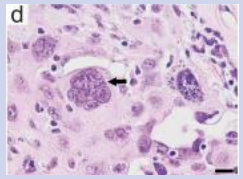
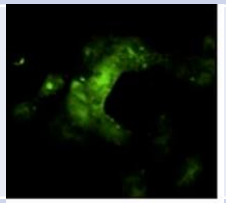
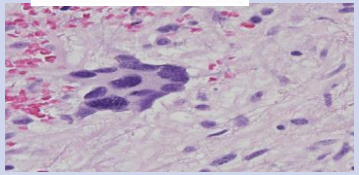
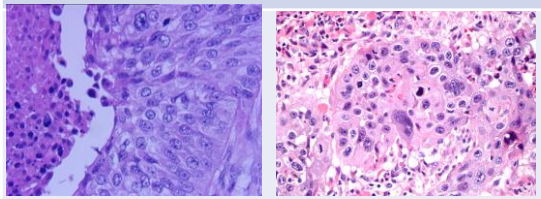








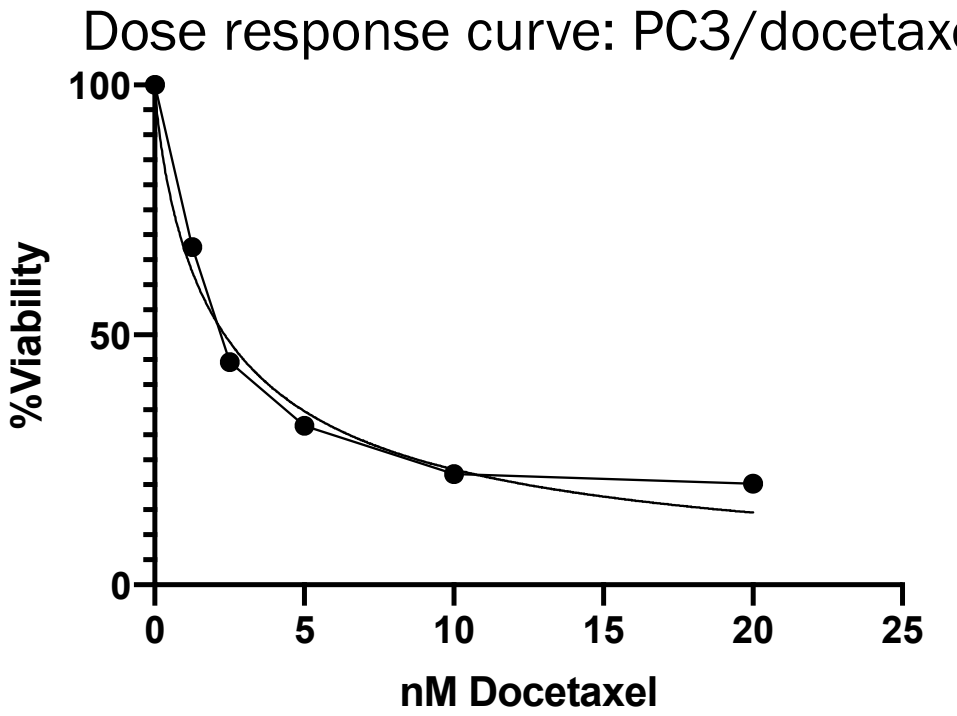
PACCs are found in multiple cancer types

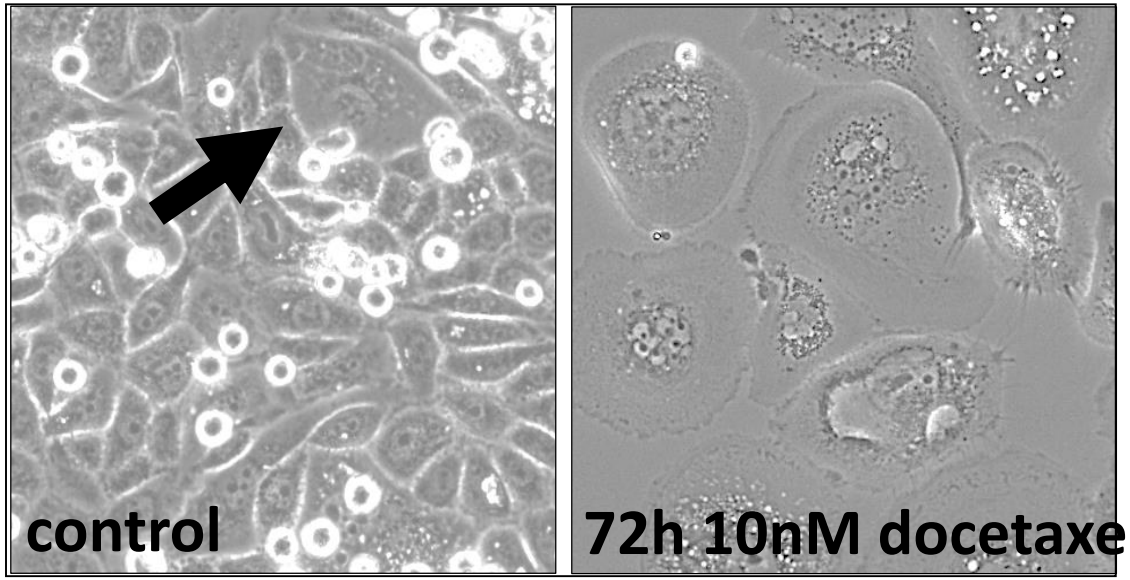
Cancer Type	Cell lines / % HACCs	Histopath
Breast	MDA-MB-231: 2% MCF7: 3%	
Colon	CACO-2: 8% HCT116: 2%	
Ovarian	SKOV3: 14% HEY-T30: 2%	
Lung	H2126: 2% H2087: 8%	
Glioblastoma	DIPG-JHU-1: 2% BT94: 4% U138-MG : 2%	
Bladder		

We know that PACCs are relevant to human cancer (not just cells grown in a lab).

What have we learned about PACCs?

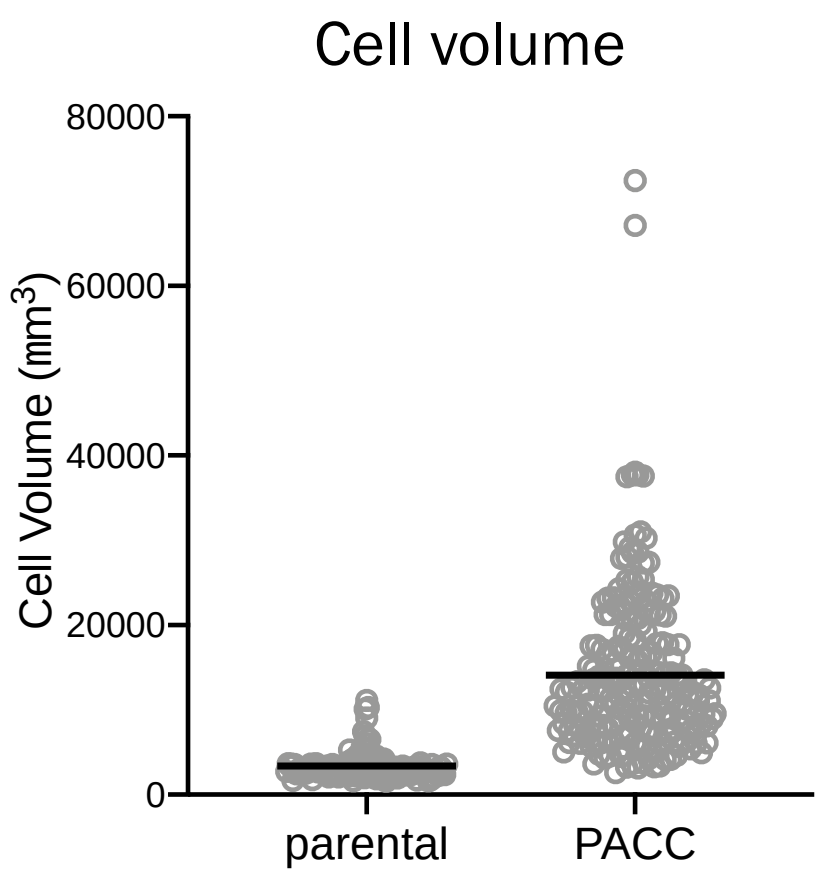
How have we missed these cells for so long?

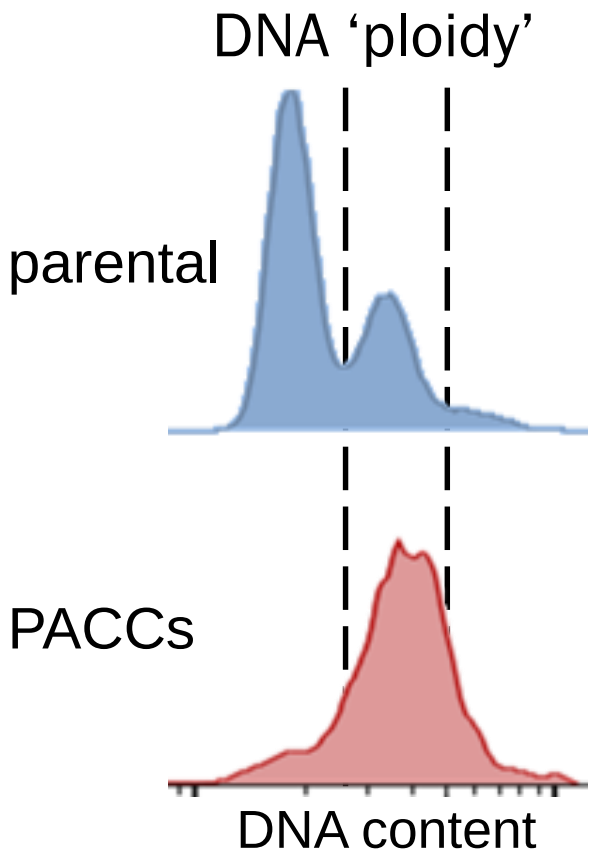




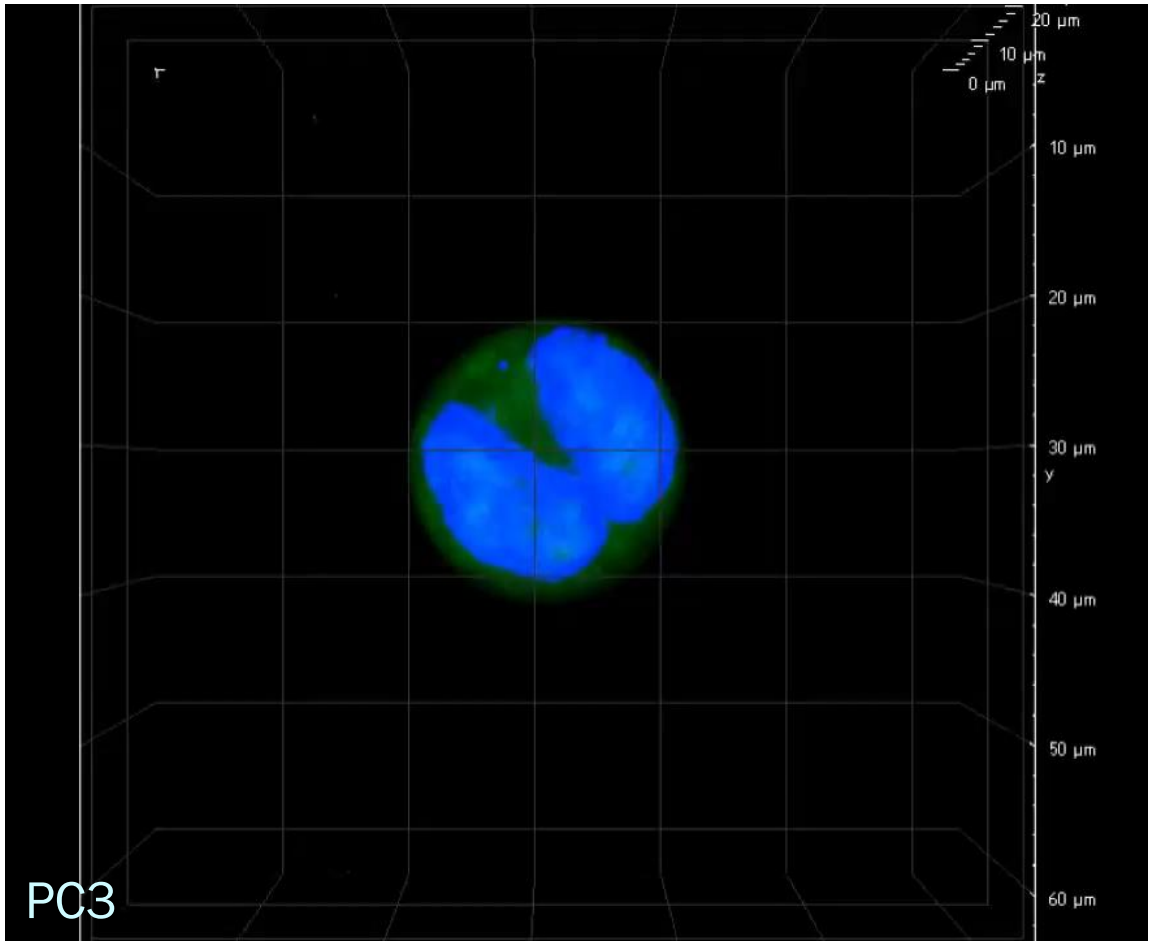
We have trained our eye to ignore them
(or we don't look at the cells at all!)

PACCs are physically larger and have more DNA than “typical” parent cancer cells

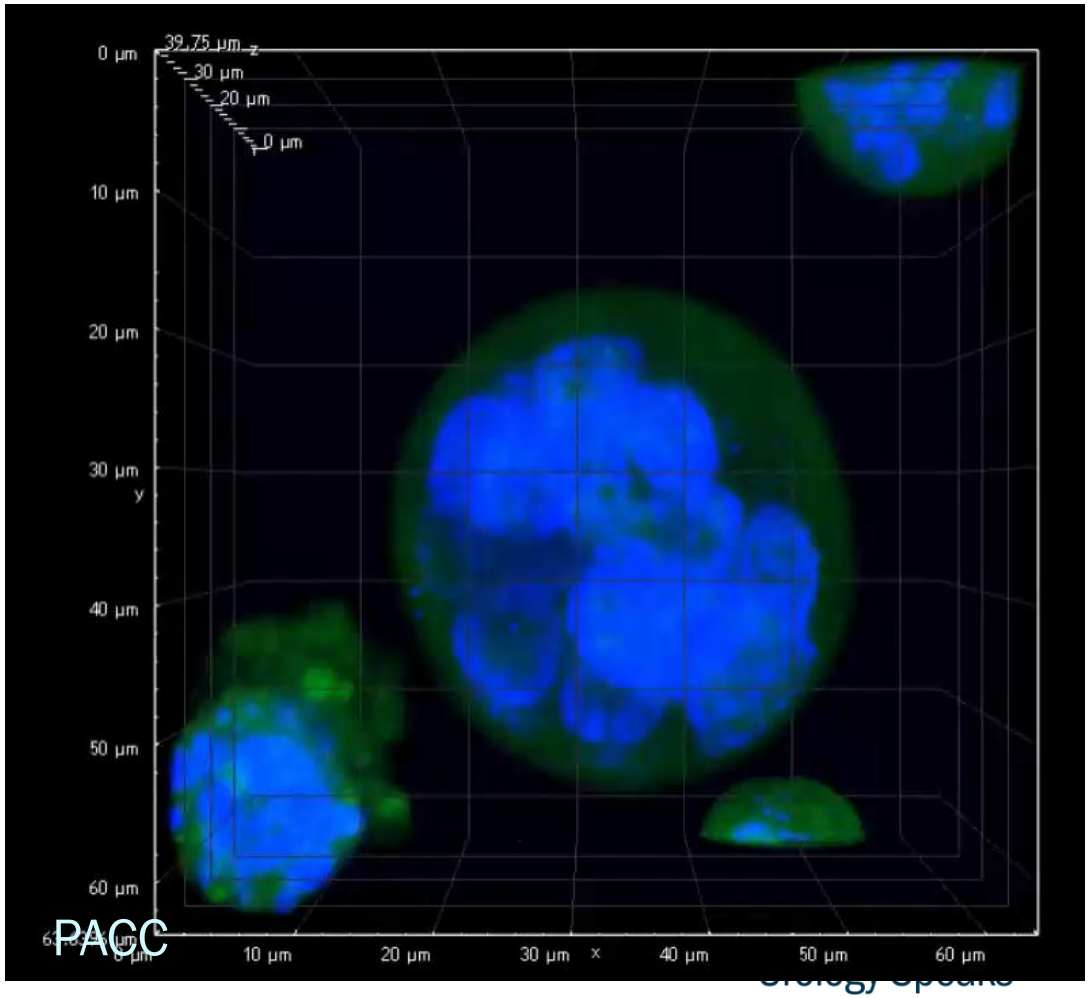




PACCs are morphologically distinct and have irregular nuclei

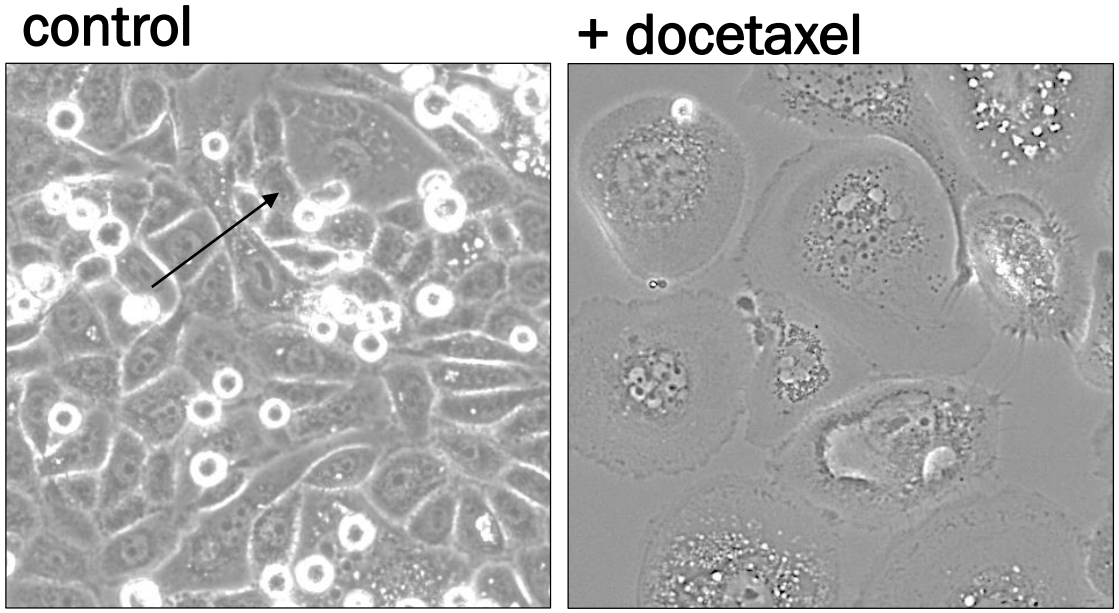


PC3



PACC

More PACCs are formed after treatment, regardless of therapy type of cancer cell line



%PACCs after 72h treatment

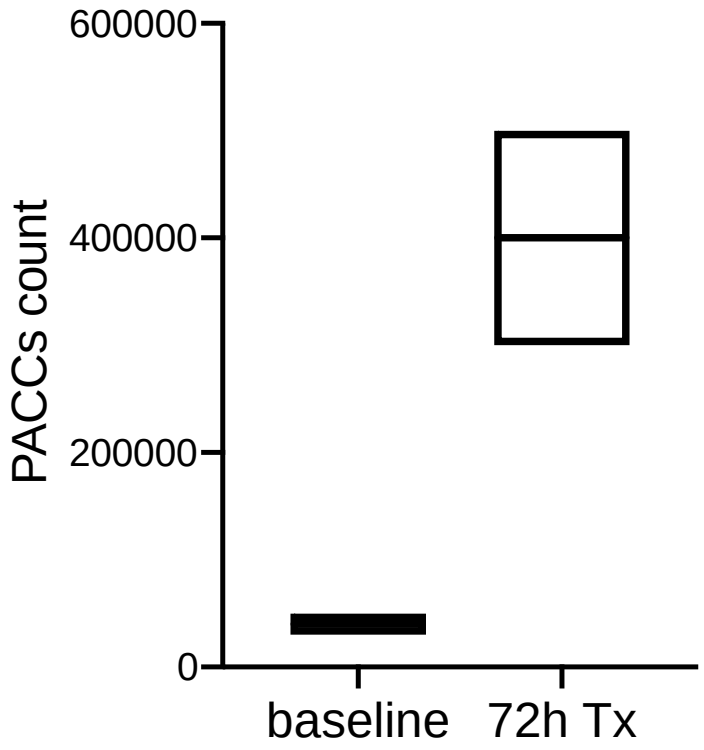
		PC3	DU145	LNCaP
Docetaxel	0 nM	3.9%	2.7%	2.9%
	0.1 nM	10.2%	19.2%	4.75%
	1 nM	13.7%	43.4%	32.9%
	5 nM	35.3%	44.8%	94.8%
Etoposide	0 uM	3.2%	3.5%	2.7%
	2 uM	12.3%	82.9%	60.5%
	16 uM	18.7%	82.0%	64.9%
	50 uM	22.3%	80.1%	78.9%
Cisplatin	0 uM	3.2%	3.6%	3.1%
	0.6 uM	4.2%	31.9%	6.3%
	5 uM	4.6%	71.8%	32.5%
	16 uM	10.8%	76.6%	67.1%

Dramatic increase in the total number of PACCs after therapy

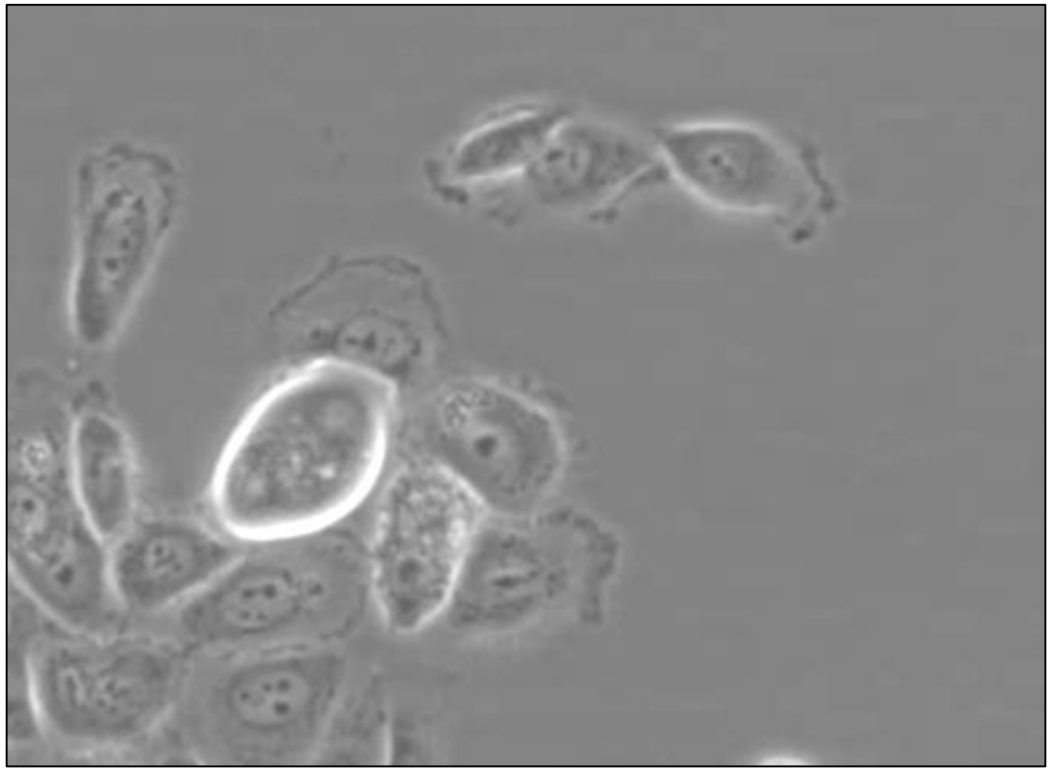
Seed 1x10^6 cells ~30,000 PACCs

Tx 72h

Harvest ~300,000 PACCs

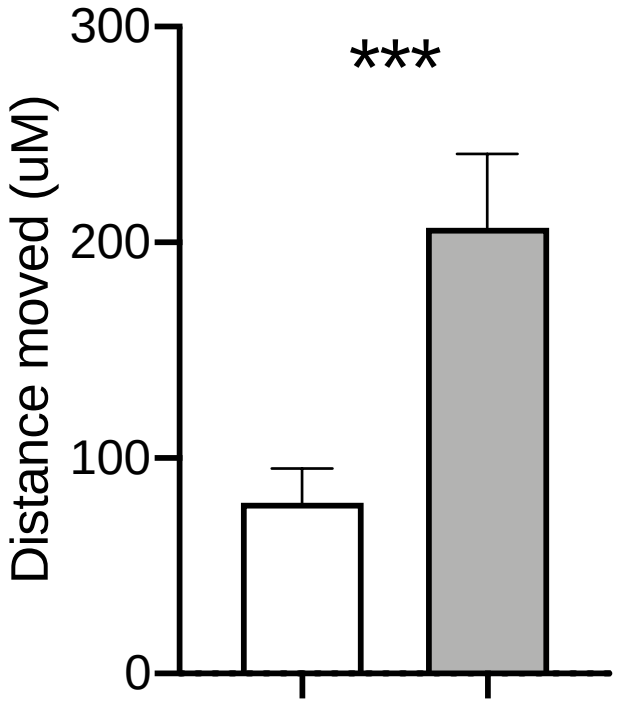


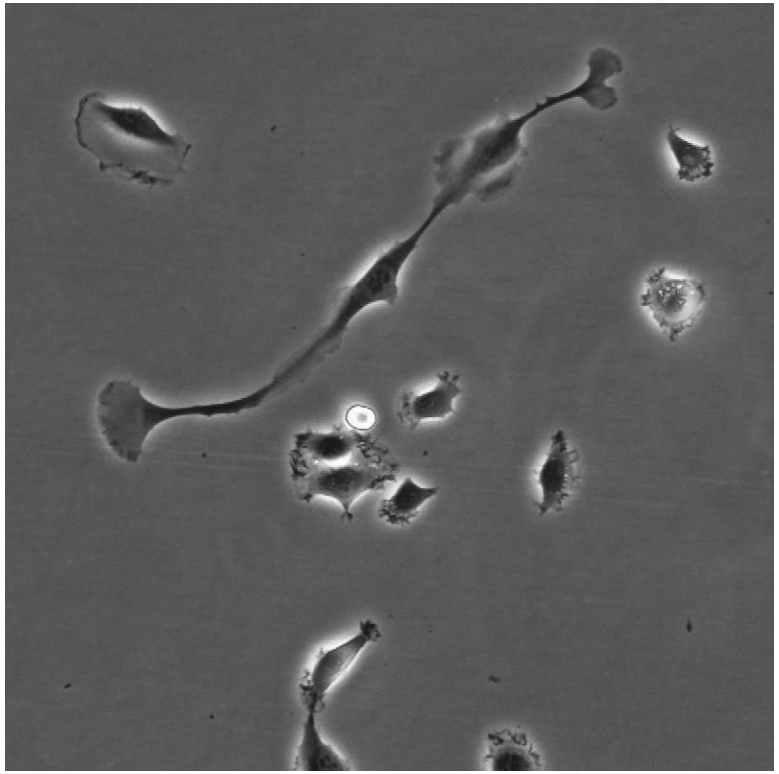
PACCs formation:
may be formed by multiple mechanisms (late endomitosis)

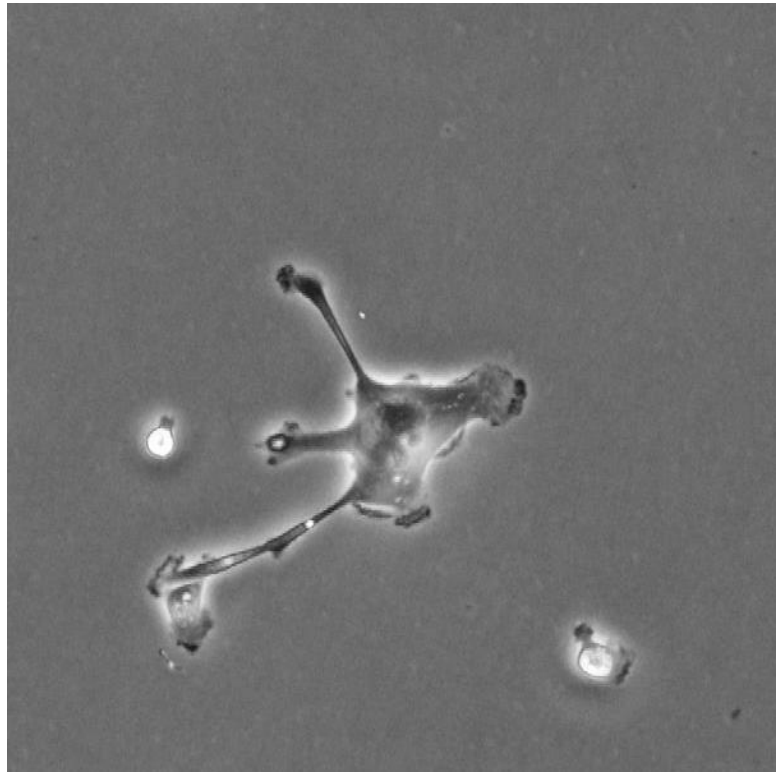


PACCs are live, active, and functional cells with increased motility

To our knowledge, we are among the first to intentionally image and track PACCs. Most drug testing is with plate-based assays.







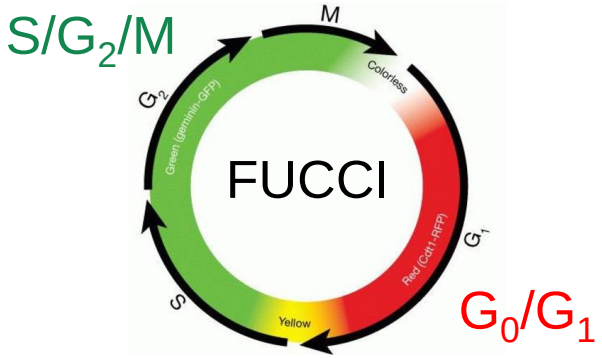
George Butler

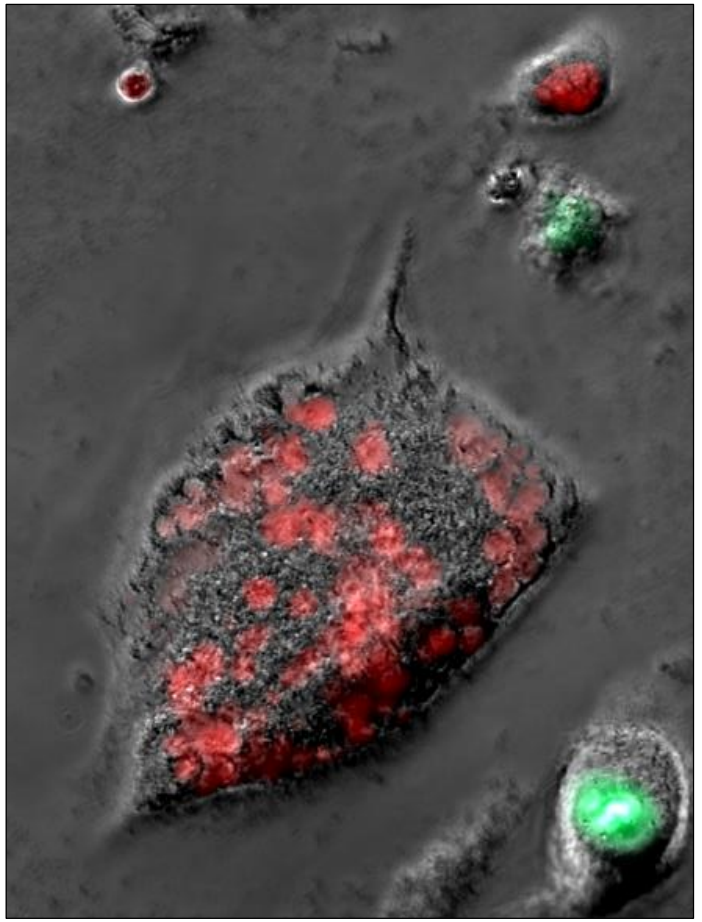
MDA-MB-231 breast cancer cells

PACCs may survive by going into “quiescence”

PC3-FUCCI cells were cultured with [LD90] docetaxel for 72 hours.

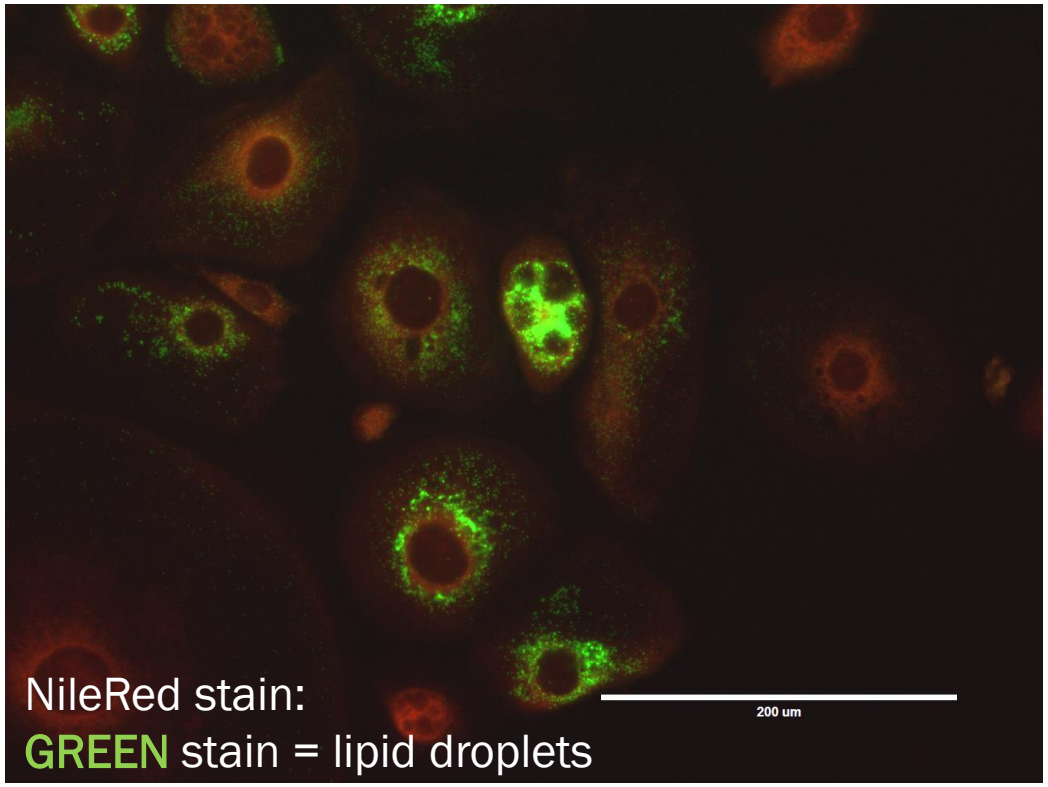
>90% of the surviving cells that continued to persist for 12 days were in G1/G0.

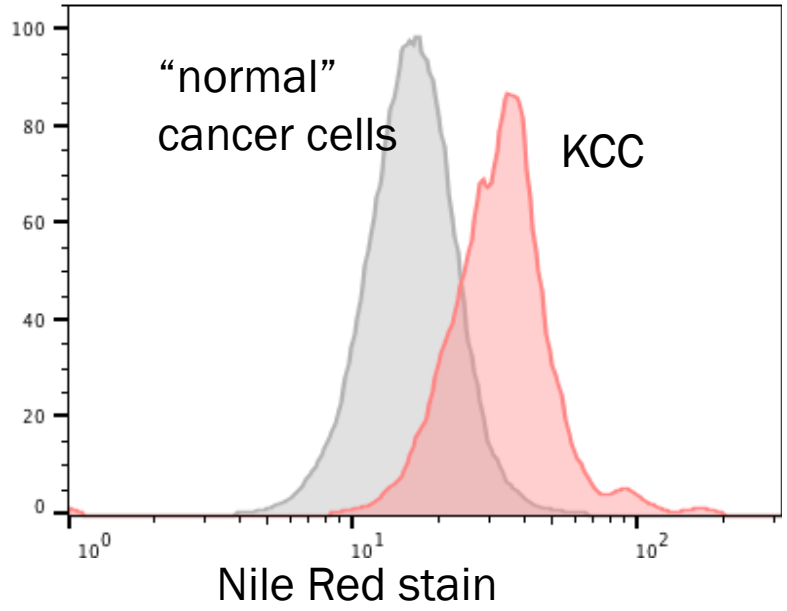




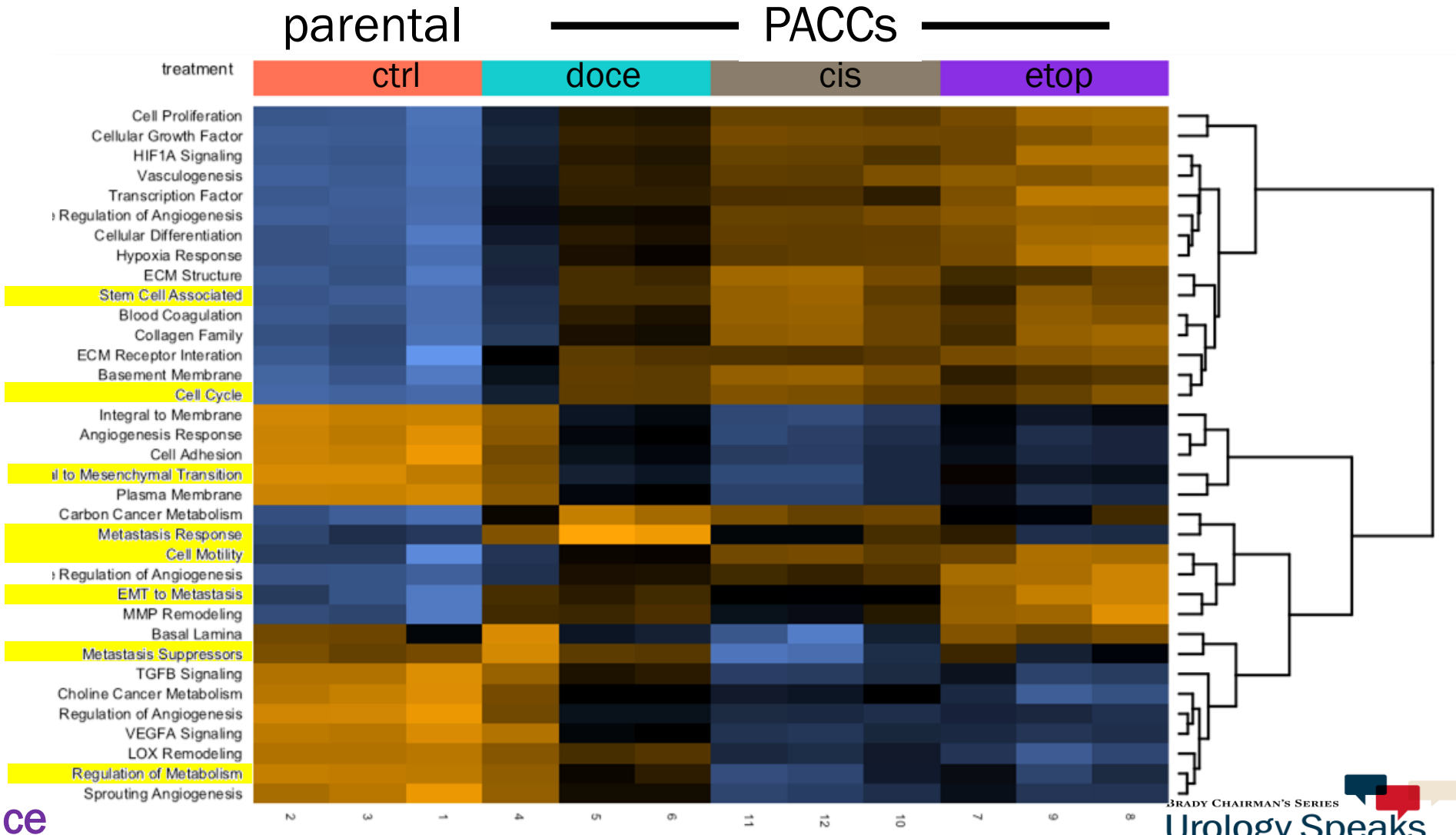
PACCs have increased fat stores: lipid droplets

PACCs likely use these fat stores to survive while stress is present





PACCs have a distinct mRNA profile



Metastasis

Therapy resistance

PACCs are resilient cells that 1) form in response to stress
2) survive therapy
3) are highly motile

KEY ATTRIBUTES:

1) Whole genome doubling
-polyploid and aneuploid
2) Exit from the cell cycle
-quiescence

How are can this be explained?

The ability to access polyploid programs enables the formation of PACCs

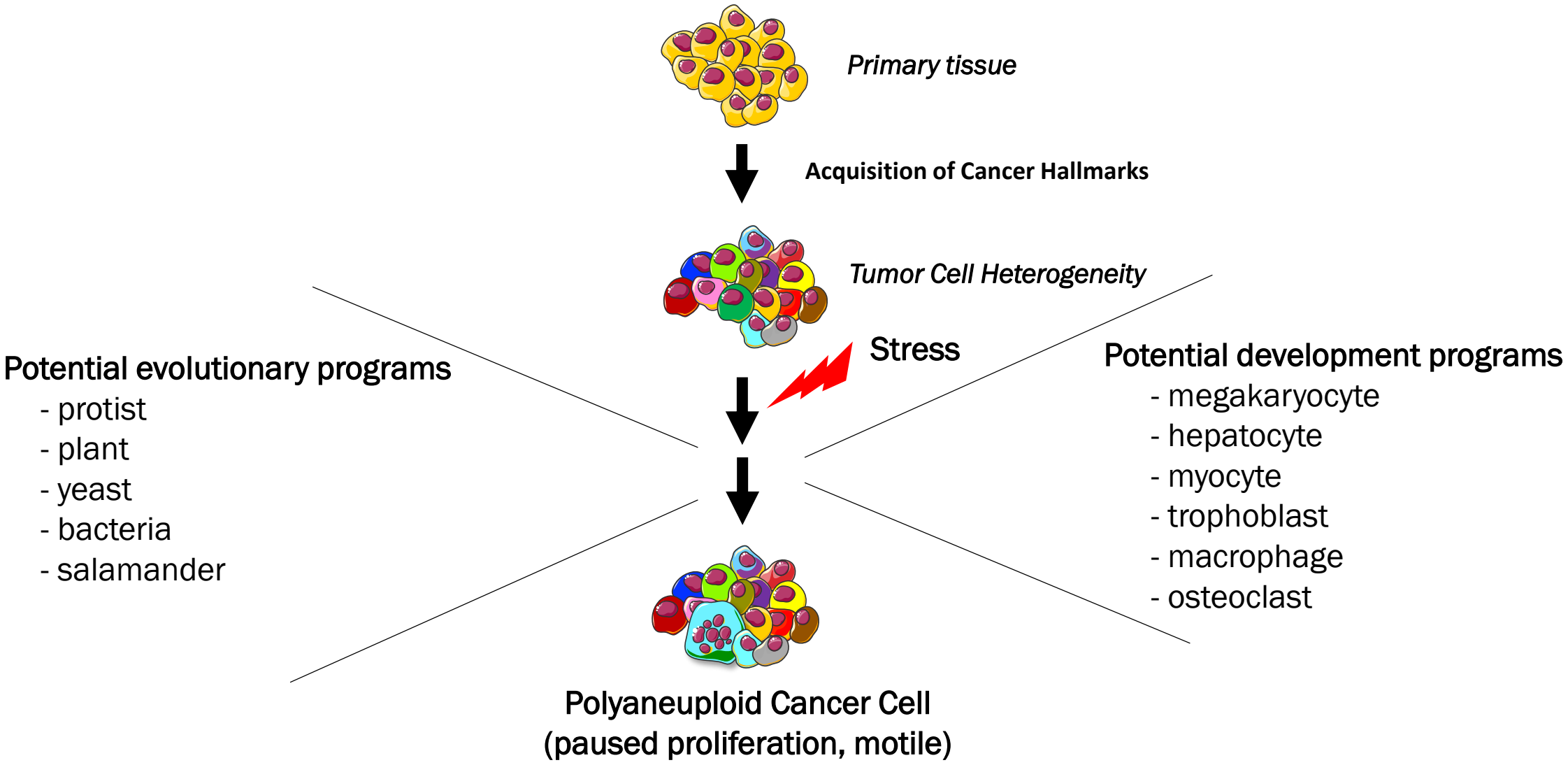


Table 1. Postulated Consequences for Polyploidization

Genomics:

1. **Increased genomic stability.** Extra copies of genes allow organisms to avoid lethal genomic damage, e.g., preventing Muller's ratchet in protists.
2. **Increased heritable variation.** The increased genomic material allows increased mutation in response to stress. Genetic instability creates progeny of various fitness allowing selection of a robust clone, e.g., antibiotic resistance in some yeast strains.
3. **Self-genetic modification.** Increased genomic material provides self-genetic modification through directed reprogramming, e.g., antibiotic resistance in some bacteria strains.
4. **New functionality.** Redundant genomic material allows mutation to achieve a new functionality. For example, two pairs of limbs allows one pair to become wings.

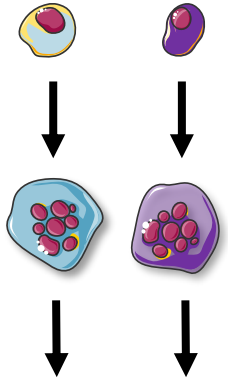
Function:

5. **Induction of quiescence.** Halting of the cell cycle leads to a non-proliferative state as a mechanism to protect the non-dividing genome while stress is present, e.g. *Entamoeba histolytica.*
6. **Increased storage capacity.** Increased cell size increases storage capacity needed for sustained quiescence (genomic material is a passenger), e.g., plant vacuoles.
7. **Increased cell function.** Increased cell size increases cell function (genomic material is a passenger), e.g., osteoclast fusion for the production of acid to lyse bone.
8. **Increased metabolic capacity.** Increased gene dosage increases production of RNA and protein products necessary for increased cell metabolism for growth, e.g., megakaryocytes.
9. **Increased toxin protection.** Increased gene dosage increases production of RNA and protein products necessary to protect from oxidative damage and cell size may protect from short term environmental toxic stresses, e.g., hepatocytes.

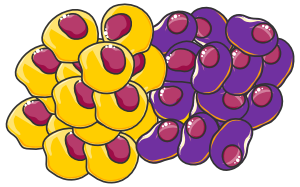
STRESS

Tumor cell heterogeneity model

Mutation present



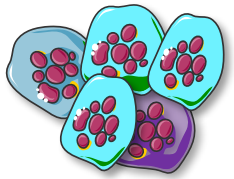
Clonal outgrowth



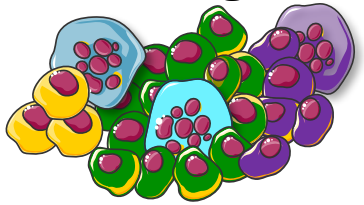
PACC formation is an obligate step of the resistance program of randomly generated and already

Quiescent state model

No mutation



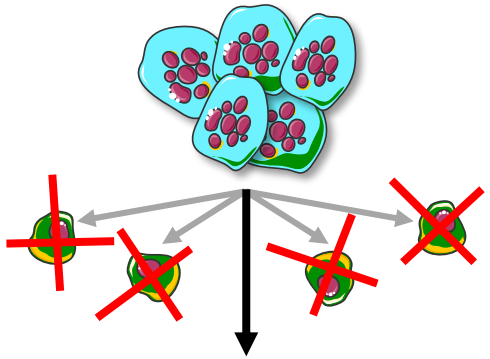
Resistant clonal outgrowth



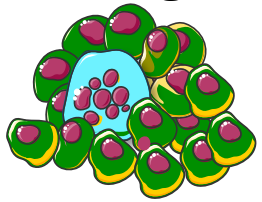
Increased genomic material is a passenger: the quiescent state is the resistant clone.

Evolutionary triage model

mutation selected



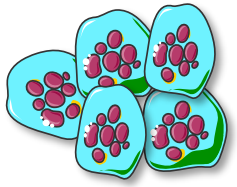
Resistant clonal outgrowth



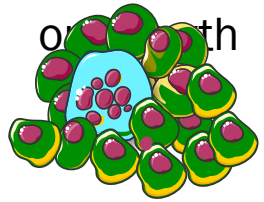
Increased genomic material allows *random* rearrangements to find resistant clone.

Self genetic modification model

mutation generated



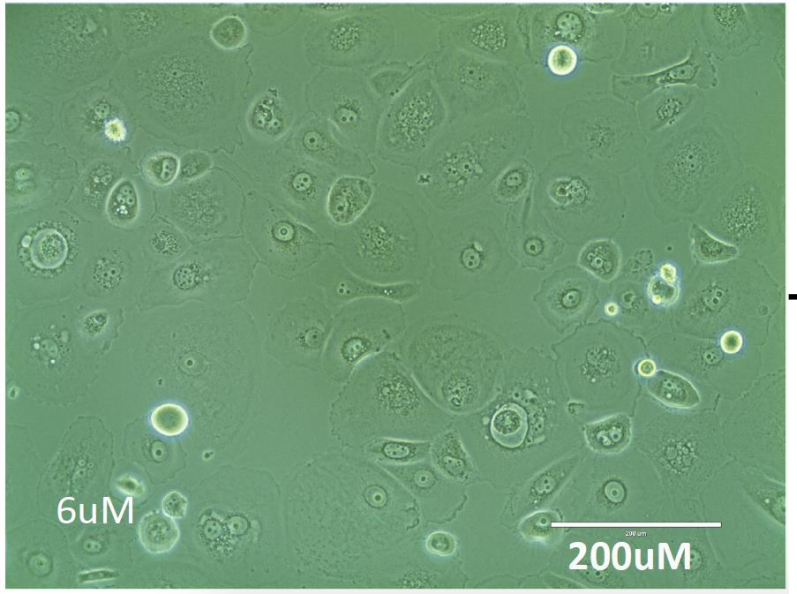
Resistant clonal o th



Increased genomic material allows *directed* rearrangement to generate the resistant clone.

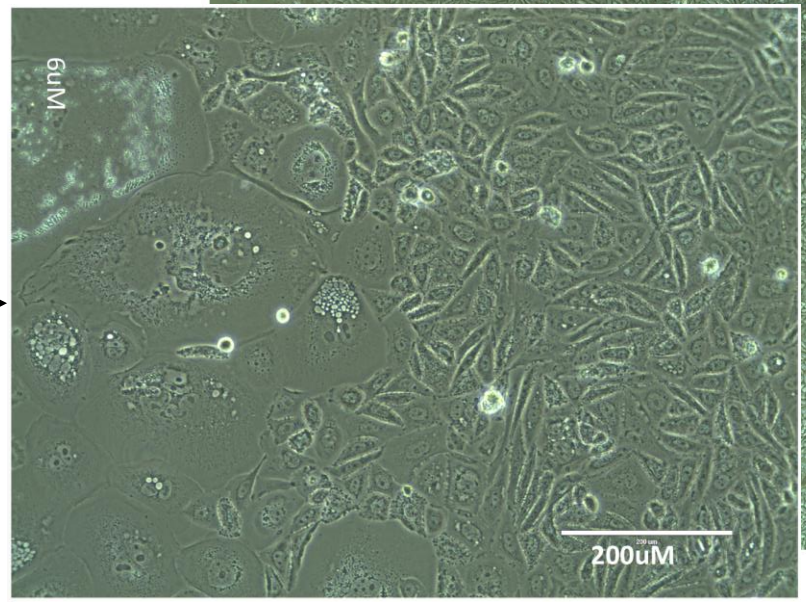
PACCs can give rise to a "recurrence" of typical-sized cells

PACCs induced w/ Cisplatin



75 days

recurrence



Metastasis

Therapy resistance

PACCs can give rise to a “recurrent” population with typical DNA amounts

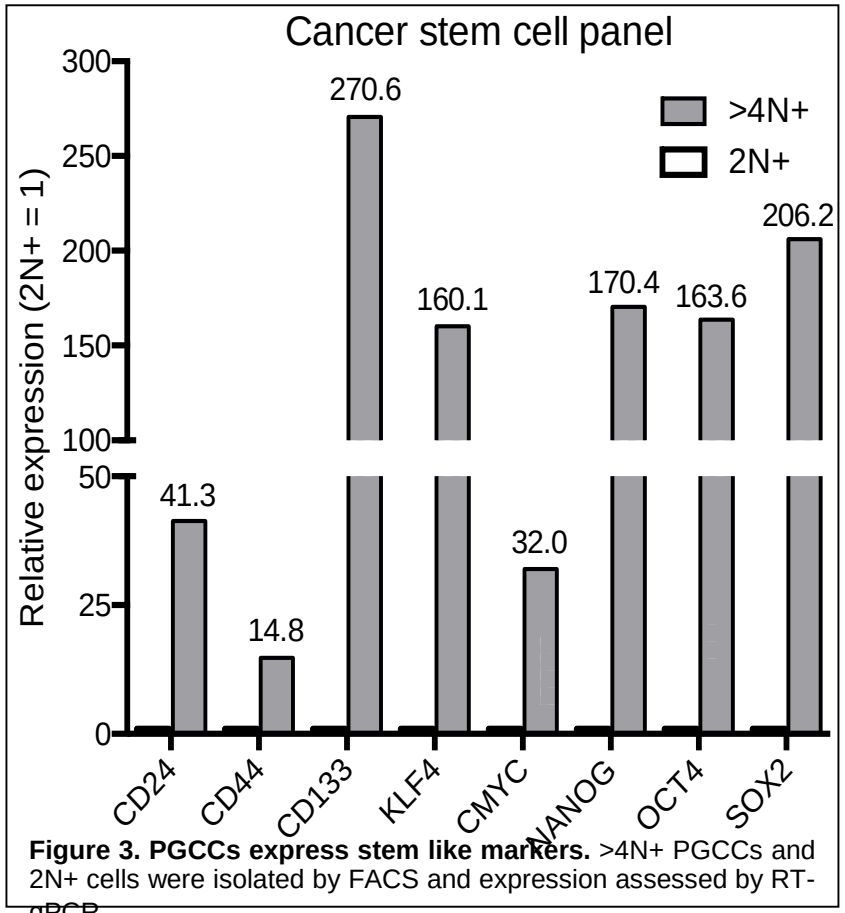
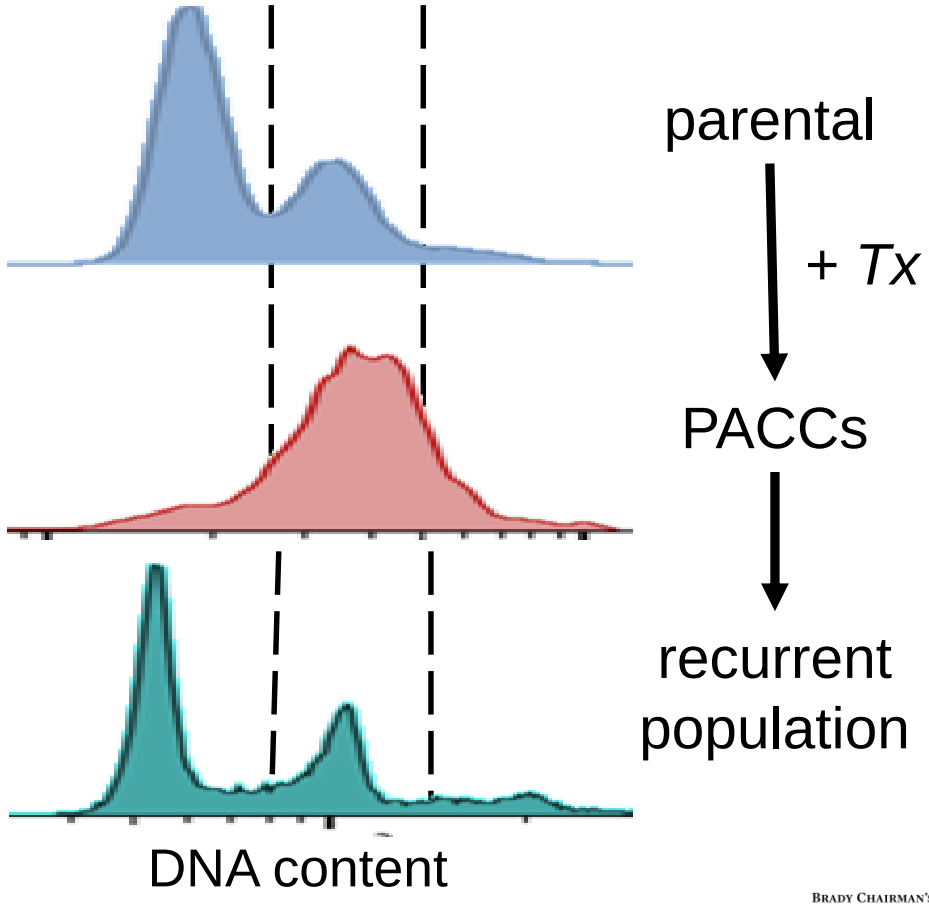
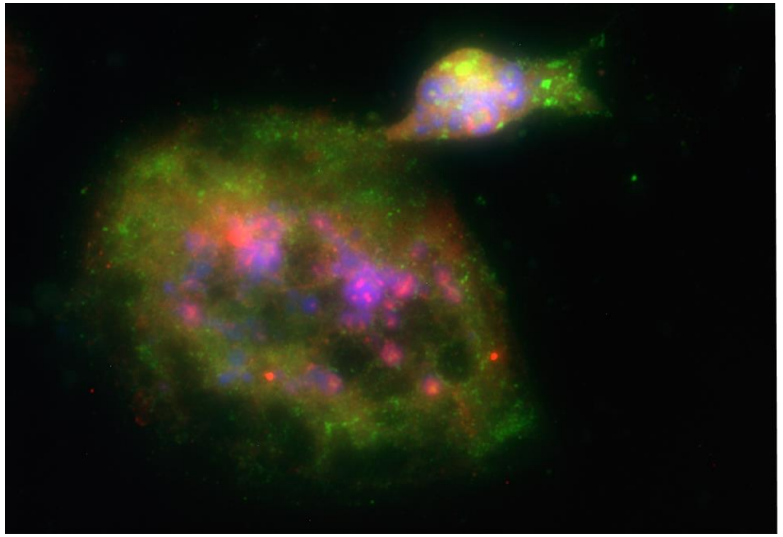


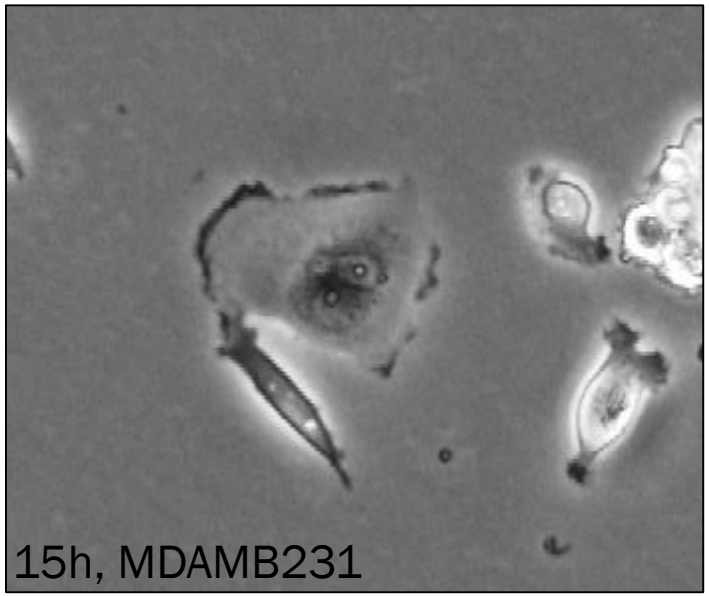
Figure 3. PGCCs express stem like markers. >4N+ PGCCs and 2N+ cells were isolated by FACS and expression assessed by RT-qPCR.



PACCs may repopulate through multiple mechanisms

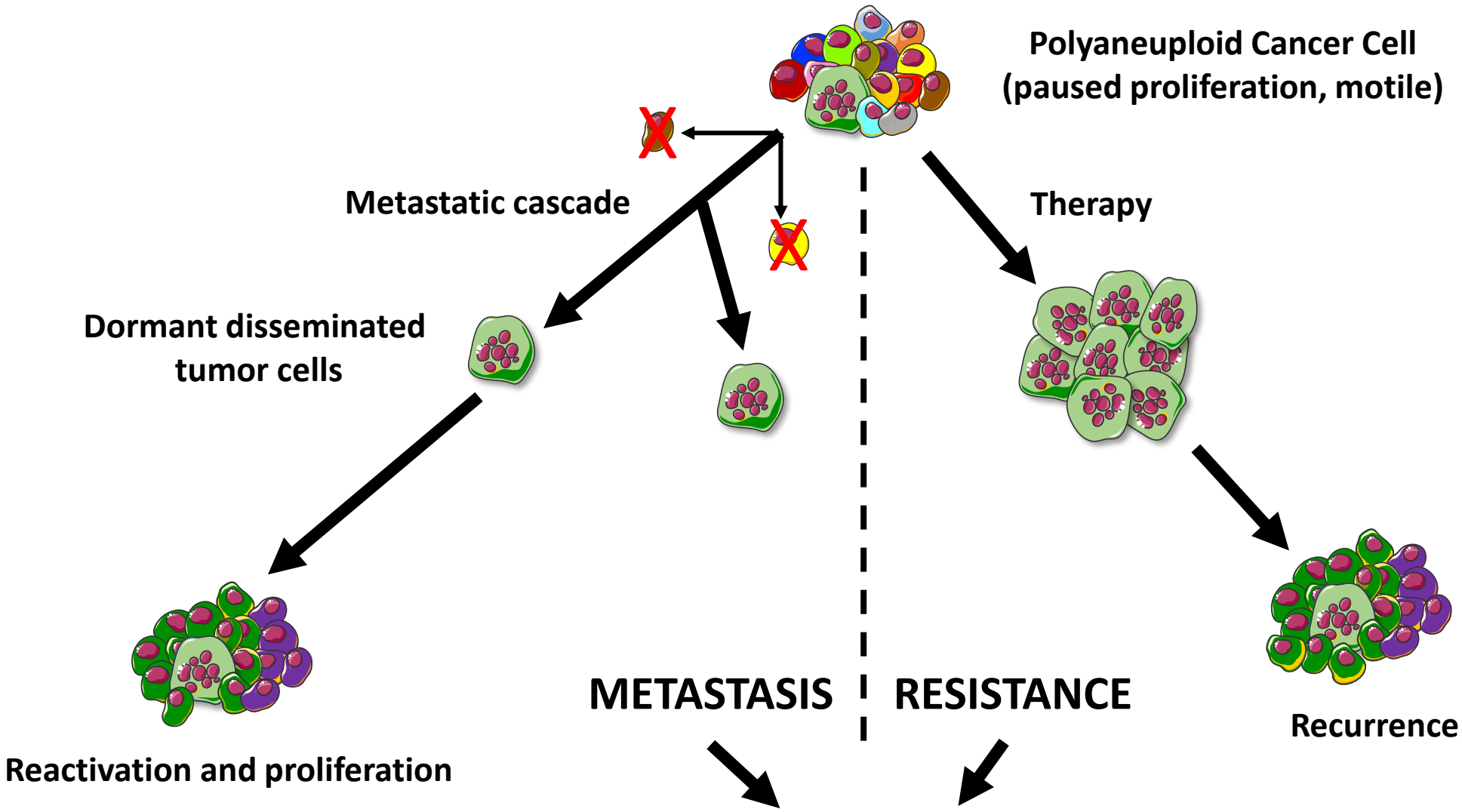


Neosis

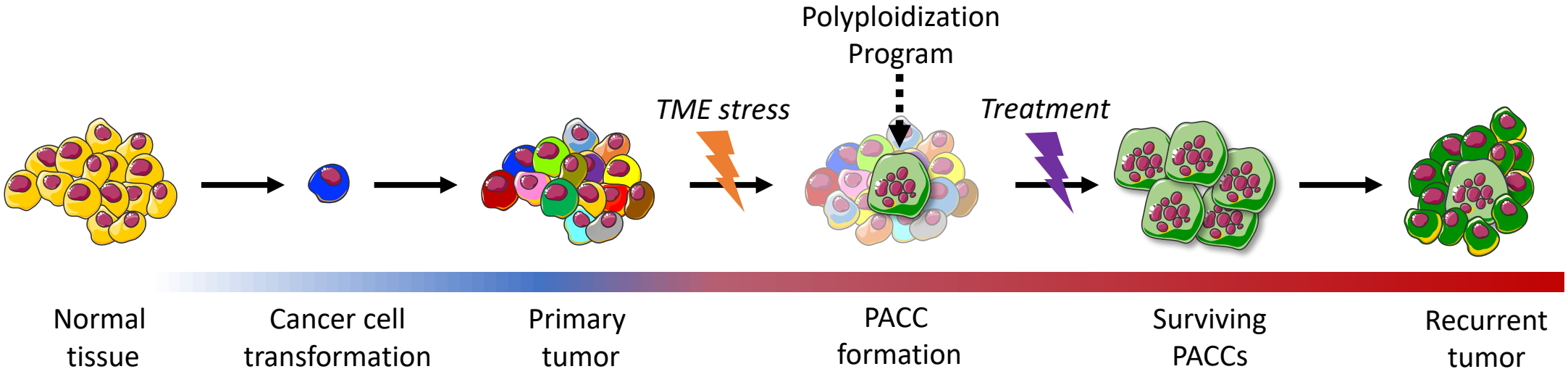


Asymmetric division

Divide from PACC to typical size and DNA amount


Polyaneuploid Cancer Cell
(paused proliferation, motile)
Metastatic cascade
Therapy
Dormant disseminated
tumor cells
Reactivation and proliferation
Recurrence
METASTASIS
RESISTANCE
CONVERGENT LETHAL PHENOTYPE

THERAPEUTIC RESISTANCE through PACCs is a HALLMARK of LETHAL CANCER



Hallmarks of Cancer

- sustaining proliferative signaling
- evading growth suppressors
- resisting cell death
- enabling replicative immortality
- inducing angiogenesis
- activating invasion and metastasis
- avoiding immune destruction
- deregulating cellular energetics

Enabled by:

- genome instability and mutation
- tumor promoting inflammation

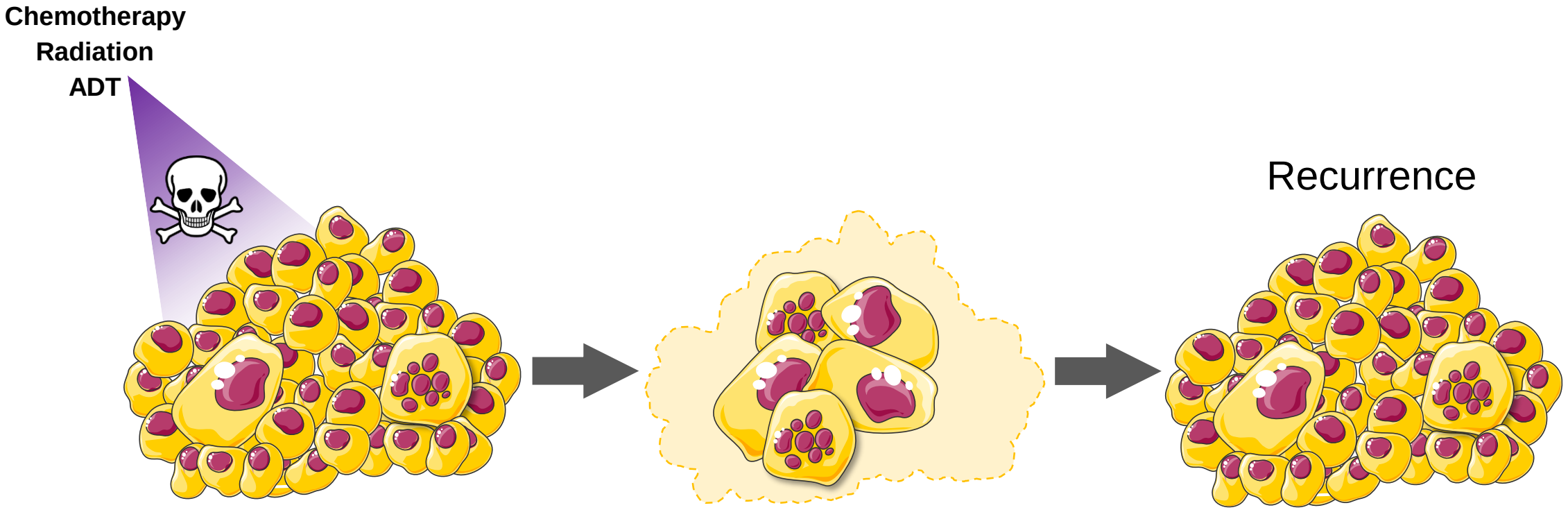
Hallmarks of Lethal Cancer

- therapeutic resistance

Enabled by:

- polyploidization
- reversible cell cycle arrest

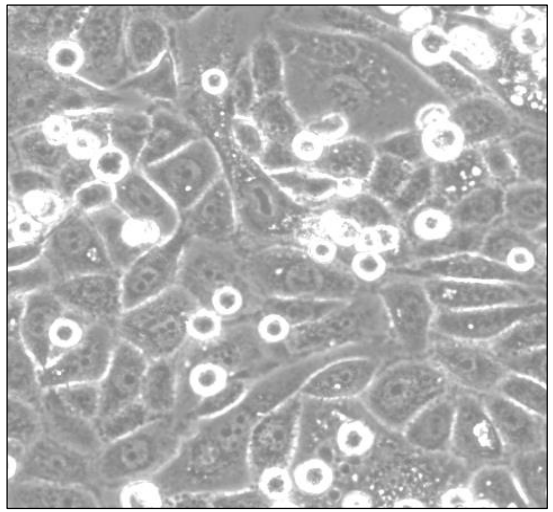
Cancer evolves resistance to all known therapy



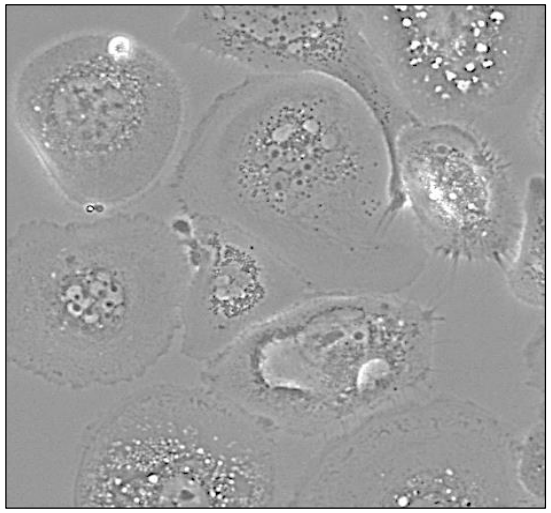
We believe that this resistance is achieved through a “PACC” phase.

Evolutionary double bind treatment strategy for cancer cure

1. Treat with cytotoxic therapy to kill the majority of the cancer cell population AND induce PACCs.
2. Treat with a therapy that eradicates the PACCs.



CHEMOTHERAPY

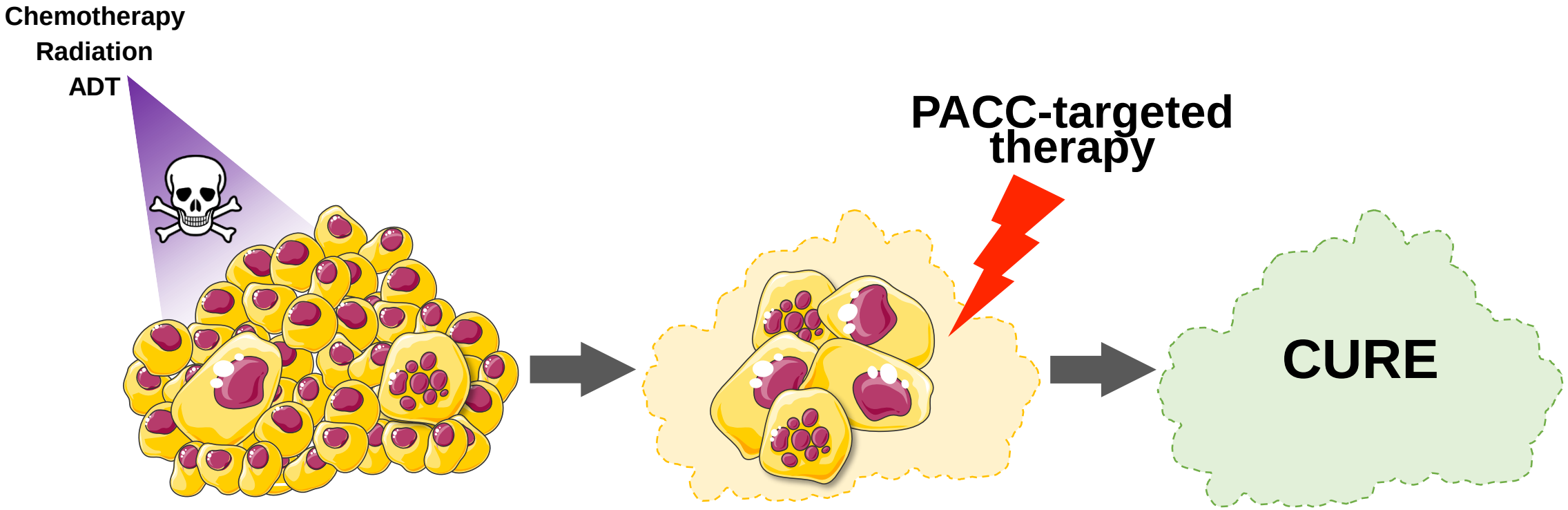


TUMOR REGROWS

THERAPY NEEDED

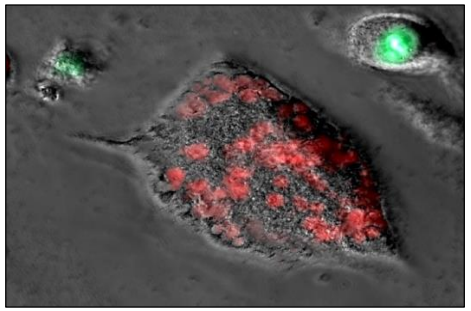
TUMOR DEAD

Apply an evolutionary double bind to cure cancer through PACC-directed therapy



1. Use traditional anti-cancer therapy to induce evolution of PACCs
2. Immediately apply PACC-directed therapy to kill the newly evolved PACCs

TARGETING PACCs OVERVIEW



- Quiescence
  - G_0
  - Senescence
    - AurK A/B
- Stem cells
  - Ag-Ab conjugates
  - ALDH1
- Aneuploidy
  - Mitotic Checkpoints
    - p53
    - Chk1
    - AurK B
  - Centrosomes
    - KIFc1
    - Centrinone
- Cell surface Antigens
  - Ag-Ab conjugates
  - IO targets
- Metabolism
  - Lipids
    - FAS
  - Autophagy
    - Chloroquine
    - Metformin
  - Glycolysis
    - AMPK
- Stress Response
  - GSH
  - Metals
    - deferoxamine
  - NAC
  - Ca++
    - amlodipine
- Protein Homeostasis
  - Protein degradation
    - bortezomib
  - HSPs
- Drug Resistance
  - PgP

Experimental agents

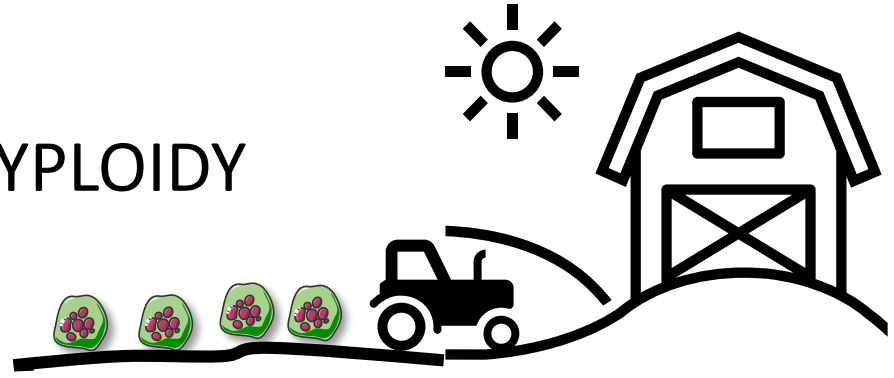
Approved drugs

STEM CELLS

SENESCENCE

WGD / POLYPLOIDY

ANEUPLOIDY



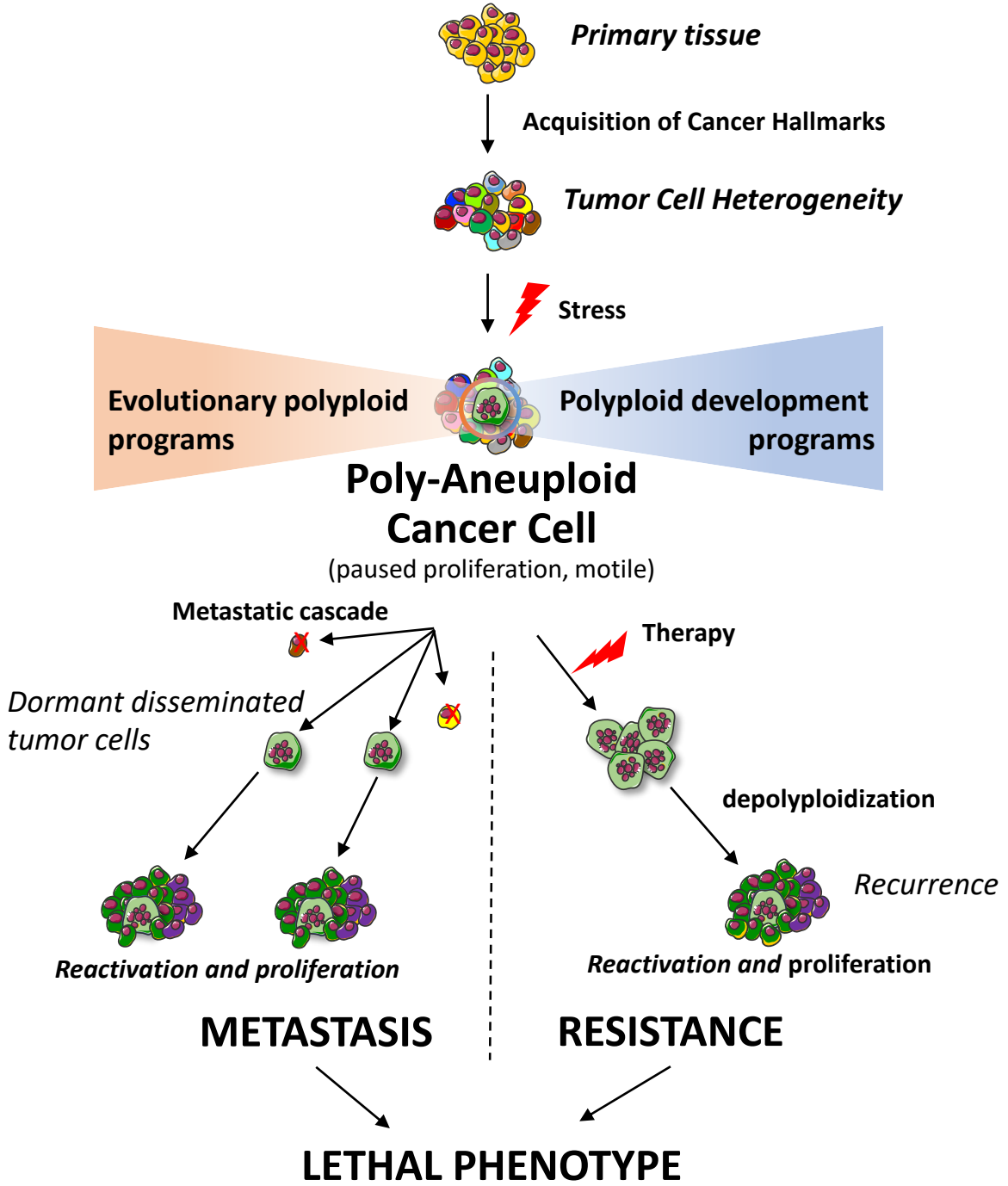
THE PACC CO-OP

METASTASIS

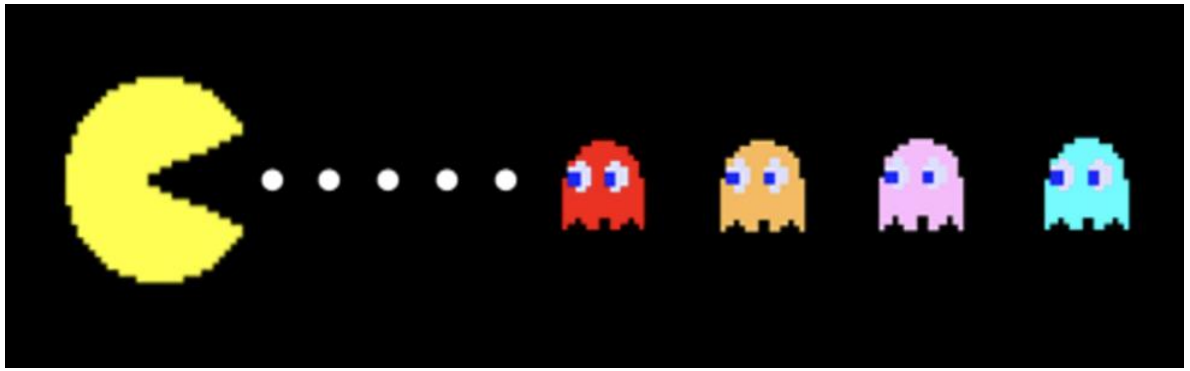
QUIESCENCE

RESISTANCE

STRESS RESPONSE


Primary tissue
Acquisition of Cancer Hallmarks
Tumor Cell Heterogeneity
Stress
Evolutionary polyploid programs
Polyploid development programs
Poly-Aneuploid Cancer Cell
(paused proliferation, motile)
Metastatic cascade
Dormant disseminated tumor cells
Reactivation and proliferation
METASTASIS
Therapy
depolyploidization
Recurrence
Reactivation and proliferation
RESISTANCE
LETHAL PHENOTYPE

Our Super-PACC



Sarah Amend
Laurie Kostecka
Mikaela Mallin
Athen Olseen
Morgan Kuczler
Chi-Ju Kim
Kayla Myers
Liang Dong
Richard Zieren

Bob Austin
Bob Axelrod
Joel Brown
Emma Hammarlund
Don Coffey

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