Is it possible to reconstruct the first few divisions after human tumor initiation? (and why this might be important!)

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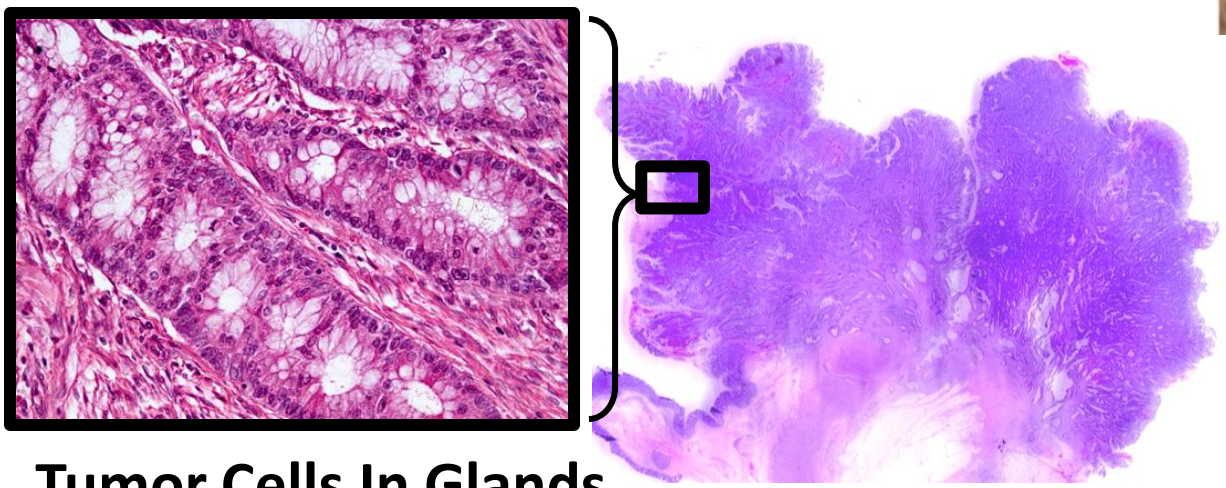
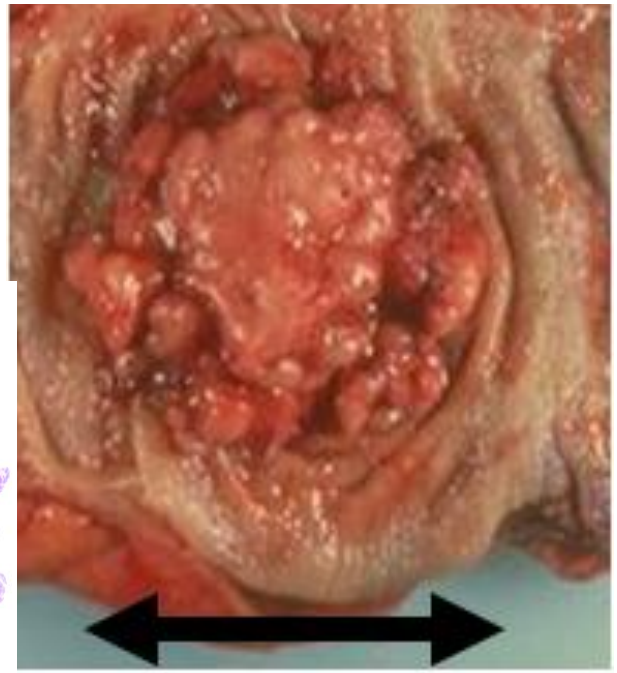


Tumor Cells In Glands
(cells have neighbors)

Adenocarcinoma



~10 billion Tumor Cells

What Happens During The First Few Tumor Cell Divisions? (tumors are clonal: all start from a single cell)

1. Nothing Special: Just Another Cell Division
2. Something "Special" (nothing normal about it)

"Special" Known: Initial Growth (both daughter cells survive and divide)

"Special" Unknowns:

--- Increased Chromosomal Instability?

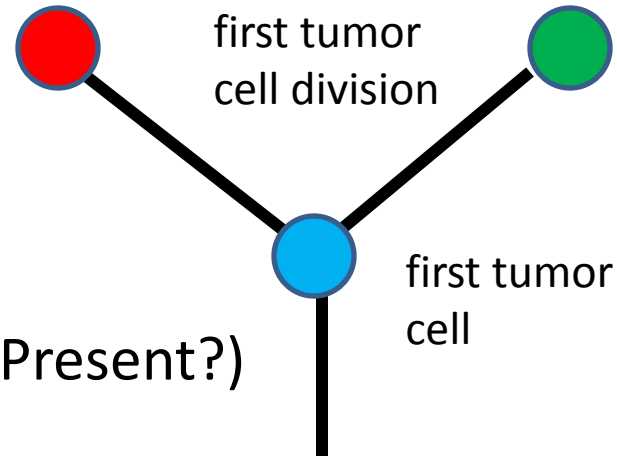
--- Increased Mutation Rate?

--- Changes in Cell Mobility?

--- Born To Be Bad?

(Ability to Invade and Metastasize Already Present?)

--- ?????

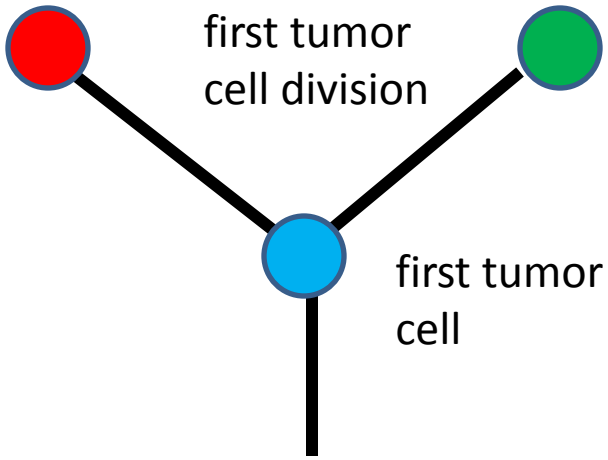


Why Is It Important To Study What Happens During The First Few Tumor Cell Divisions?

1. **Early Cancer Prevention:**
   Better Understanding Of What To "Prevent"

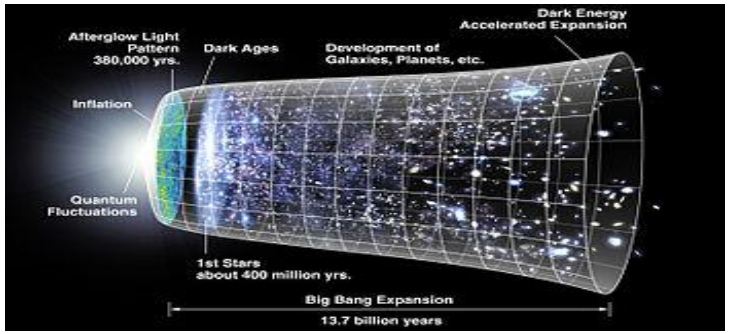
2. **Distinguish Benign Versus Malignant Small Tumors (Born To Be Bad):**
   Are The First Few Divisions Of Malignant And Benign Tumors Different?

3. **Measurable (?):**
   The First Few Tumor Cell Divisions Are "Easy" To Measure

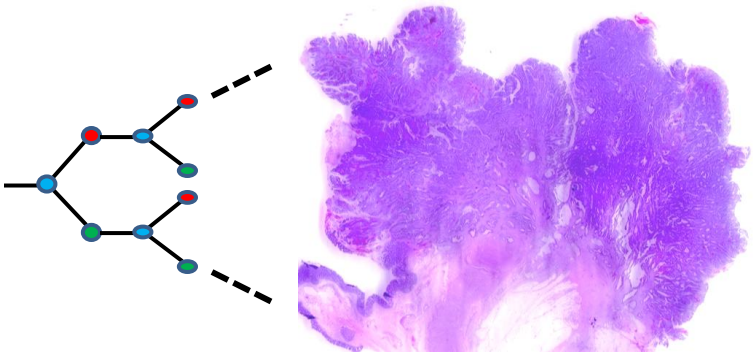


For Fun: A "Physics" Type Of "Story"

Concept	Physics	Cancer
BIG BANG	"Start" (13 billions yrs ago)	Single Cell (clonal)
QUANTUM MECHANICS	Energy In Stable Discrete Packets	Mutations In Stable Discrete States (Fixation)
Time (relativity)	Uniform Age (Speed of Light Constant)	Uniform Age (Tumor Cell Mitotic Age Constant)

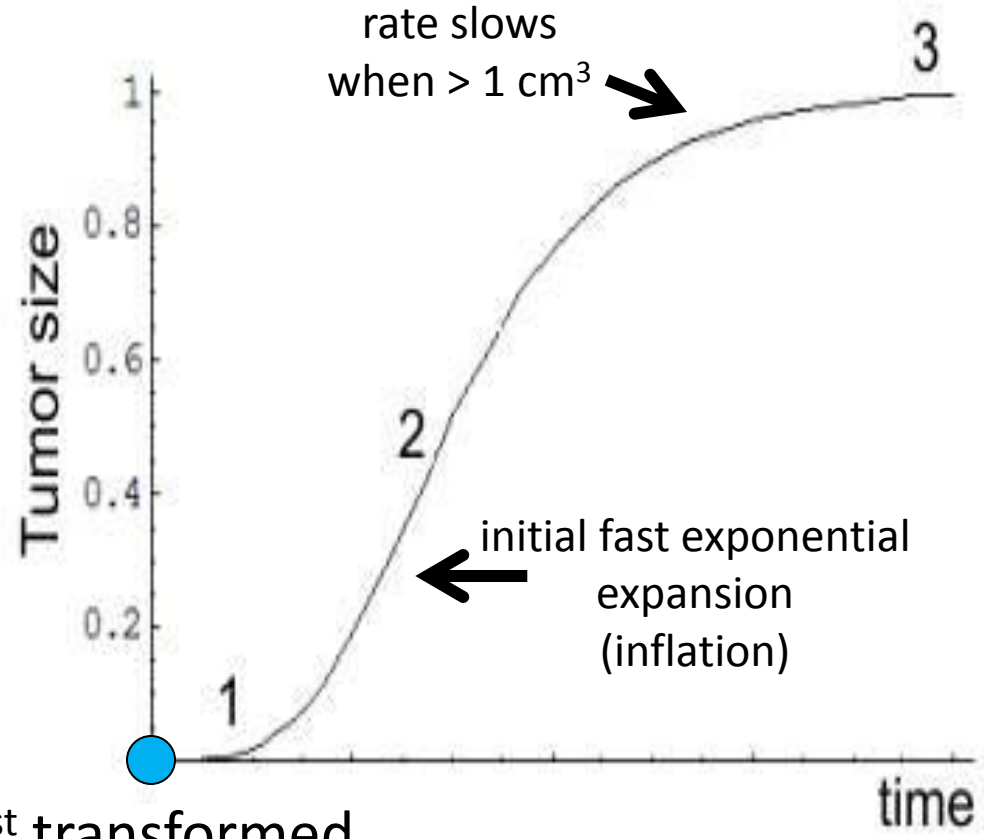


Big Bang Universe



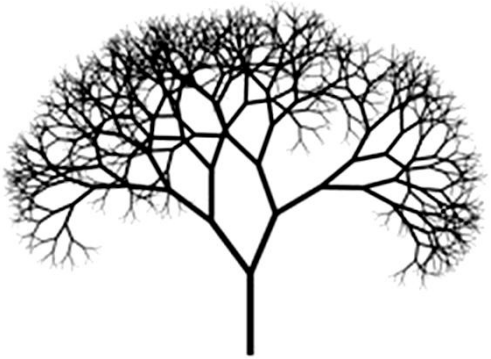
Big Bang Tumorigenesis

Observations of Tumor Growth



1st transformed cell

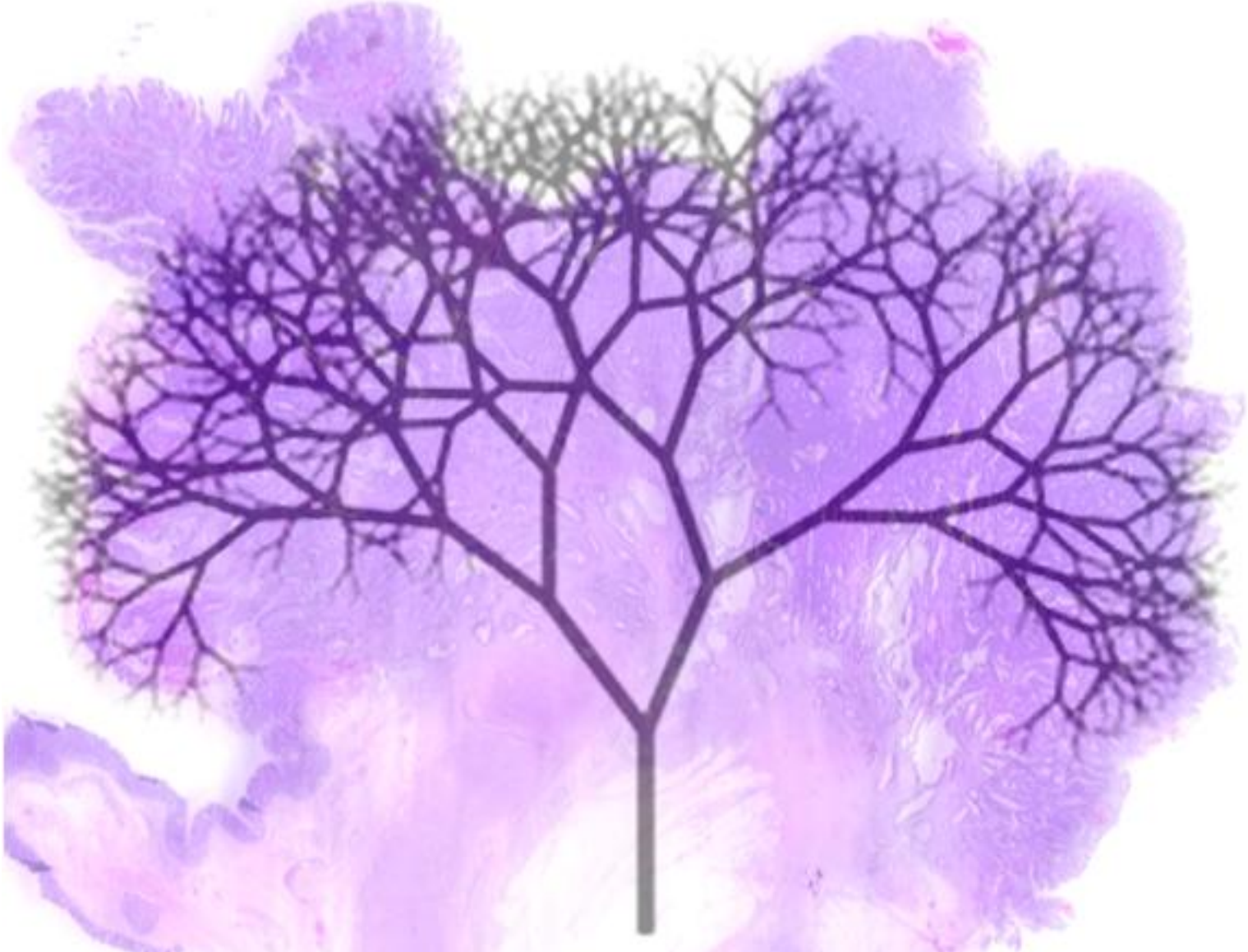
3 cm



early binary exponential division

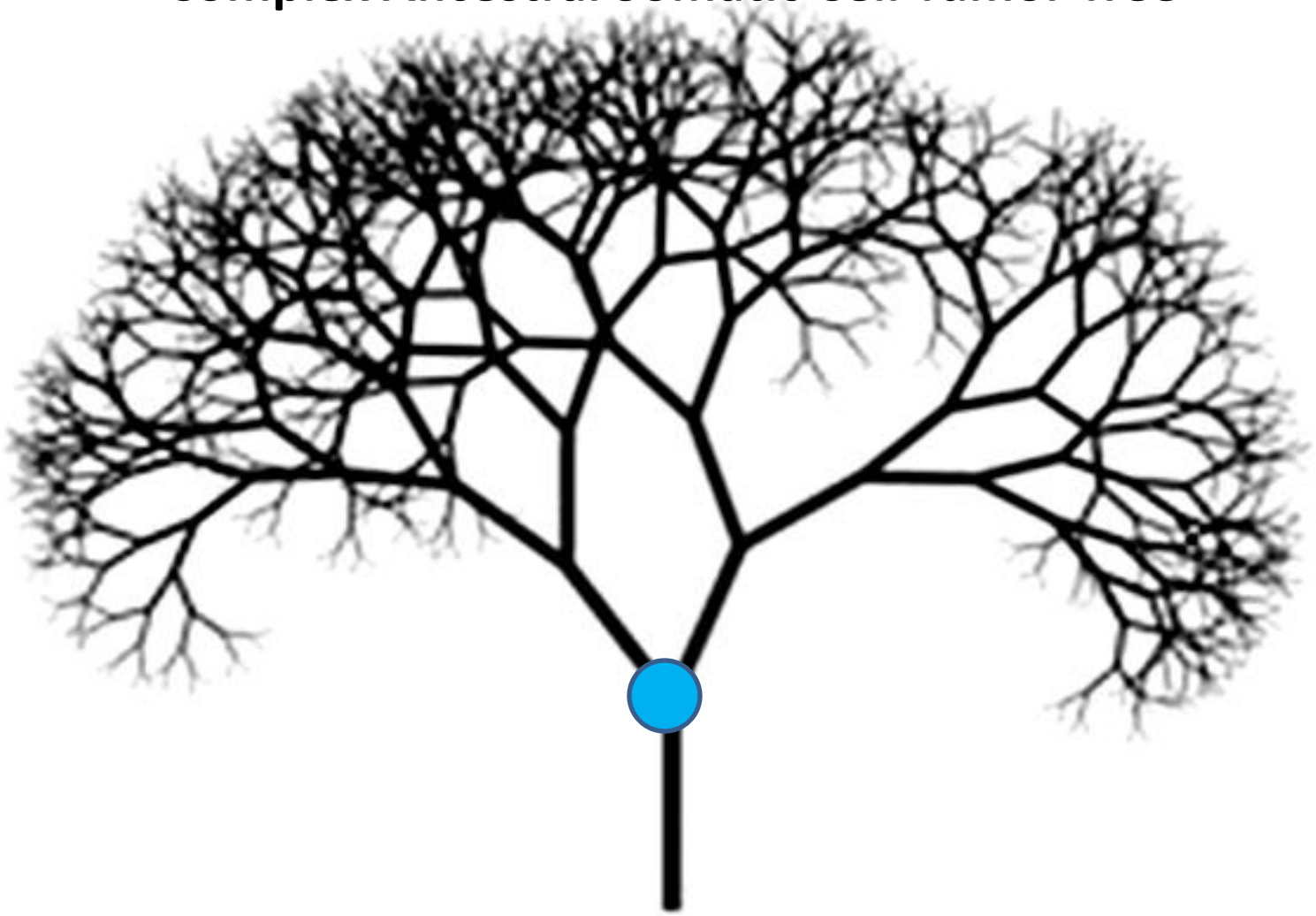
Tumors Typically Exhibit Gompertzian Growth

Complex Ancestral Somatic Cell Tumor Tree



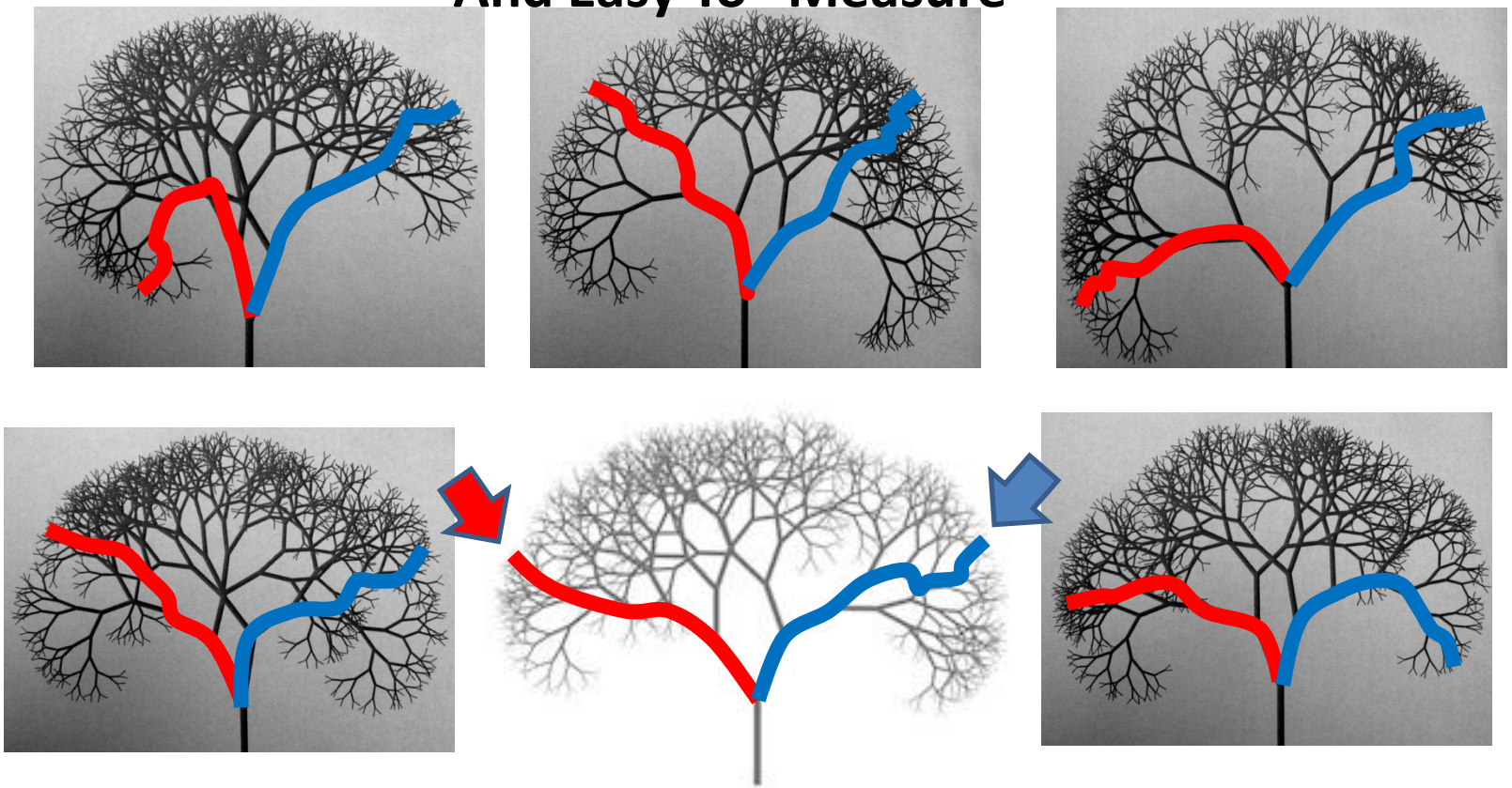
one start with billions of tips

Complex Ancestral Somatic Cell Tumor Tree



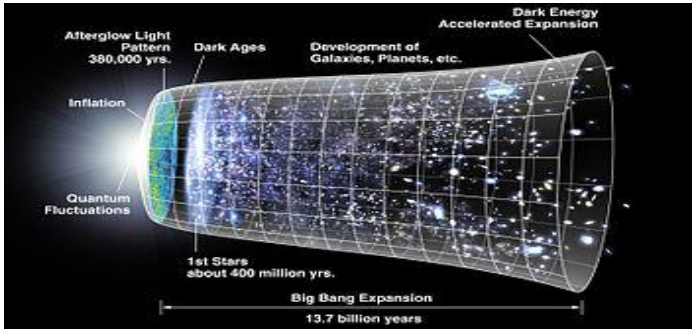
Start From Single Cell With Early Exponential Growth = Star-like Phylogeny (cells with similar mitotic ages)

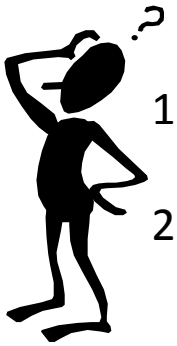
Many Possible Binary Trees:
BUT Early Tree Structure Relatively Is Simpler
And Easy To "Measure"



Sampling From "Opposite" Tumor Sides Can Identify Early Private Mutations

How To Measure The "Start" Of The Universe?

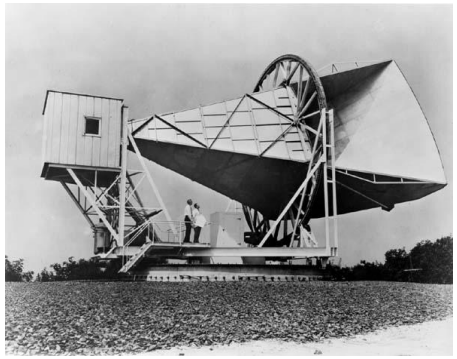




1. First Few Seconds Of The Universe Extremely Well-characterized
2. Physicists Must Be Really Smart!!!!

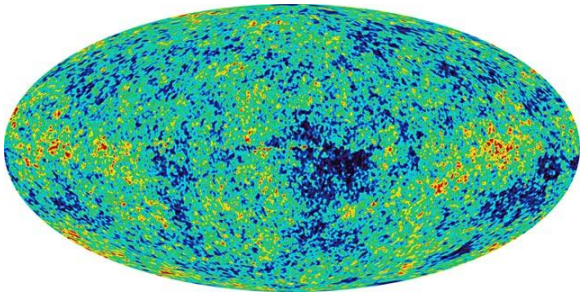
Big Bang

Alternatively: A "Start" Is Easy To Characterize Because The Signal Is "Everywhere"



Primary Evidence Is The Background Temperature Or Glow of the Universe (3 degrees Kelvin)

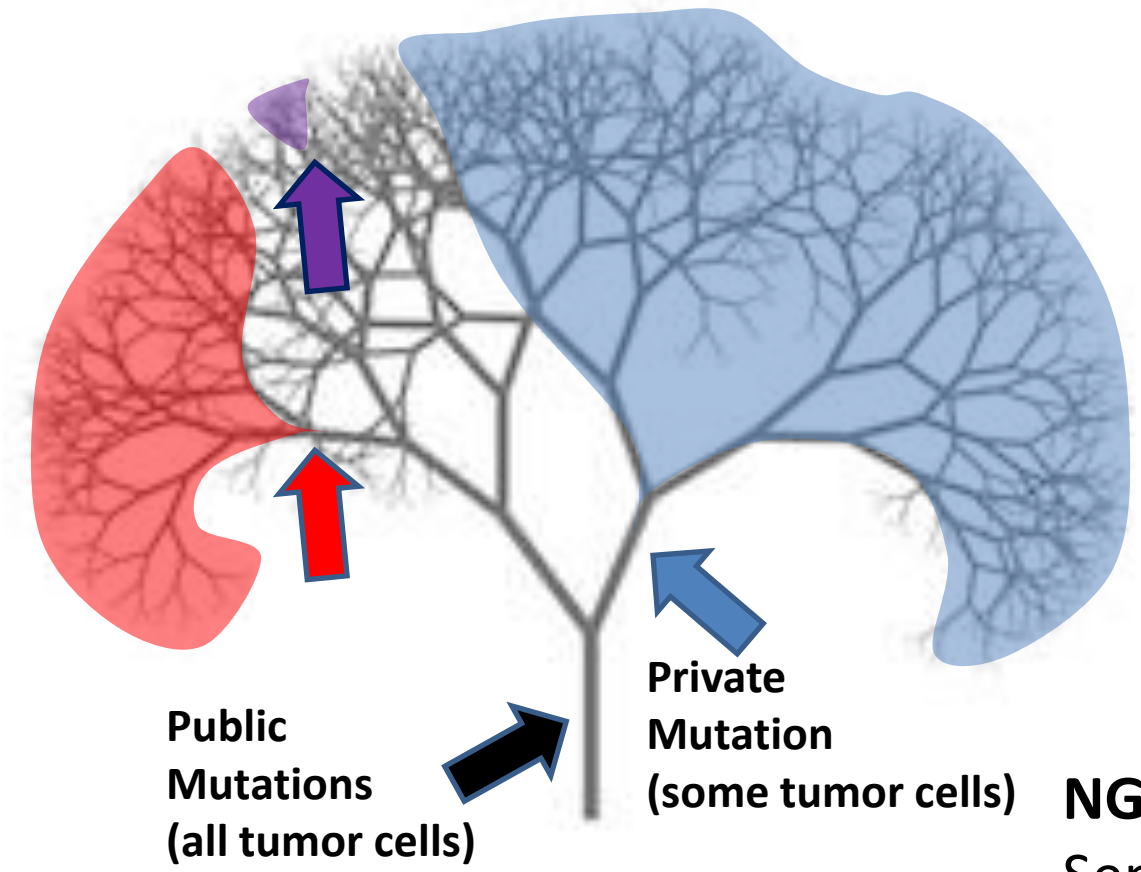
Microwave Radiation (Static) Is Uniform Or The Same In Every Direction



Uniformity Is Due To Mixing And Rapid Early Expansion (inflation)

The Tumor "Start": Signal Is Early Private Mutations

1) Easy To Sample
2) Easy To Detect



simple exponential expansion

Public: 100% cells
Private:
Division 1: 50%
Division 2: 25%
Division 3: 12.5%
Division 4: 6.25%
Division 5: 3%

NGS Platforms:
Sensitivity About 10% Mutation Frequency

Left Tumor side

Right Tumor side

private mutations

public mutations

private mutations

initiating cell

private mutations

public mutations

zygote

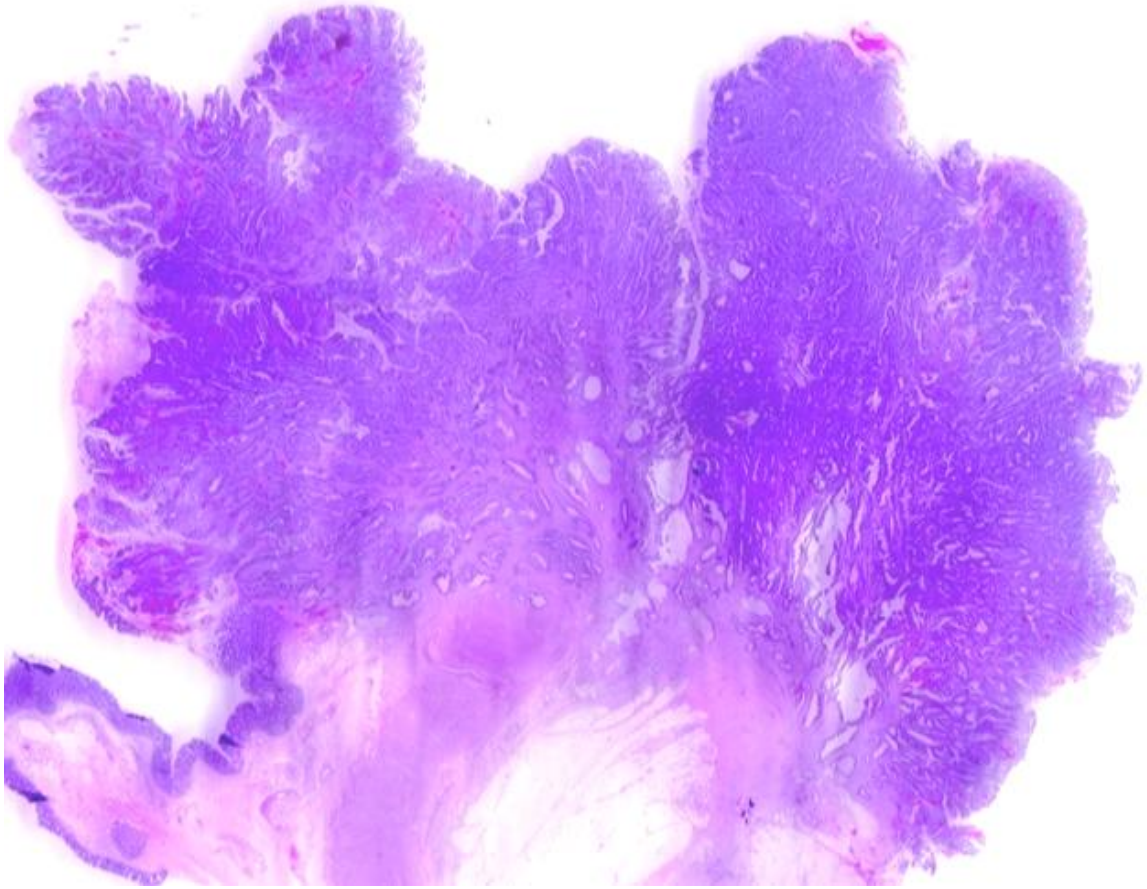
Model System: Human Colorectal Cancer

Specific Goal:

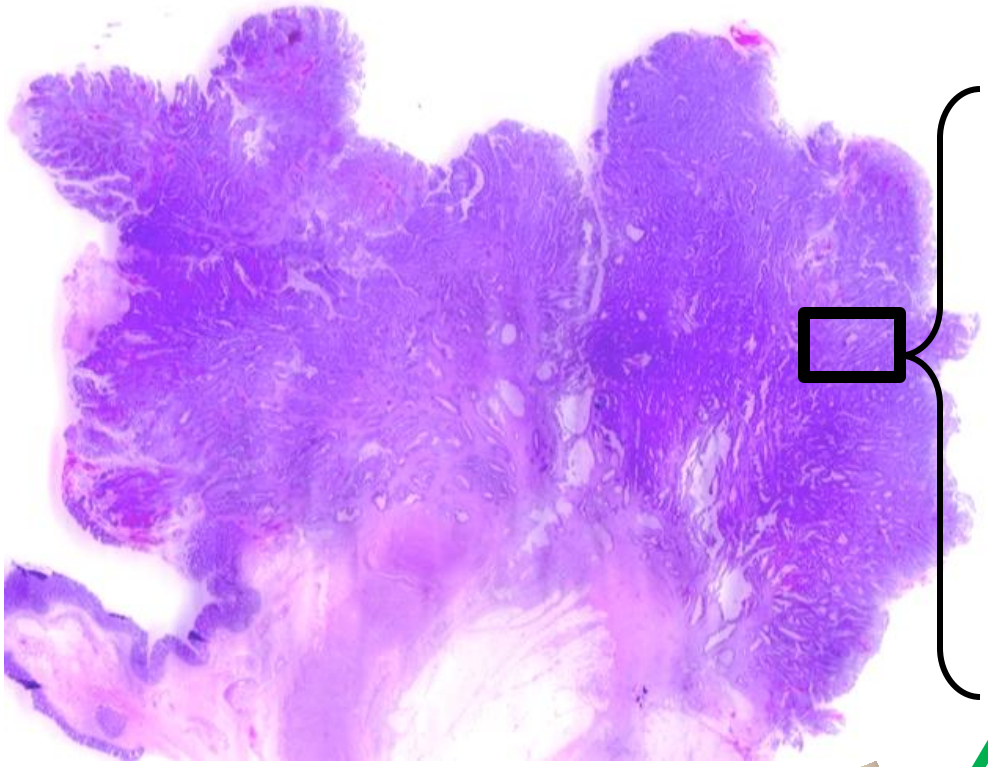
Understand Tumor “Initiation” (first few divisions after transformation)

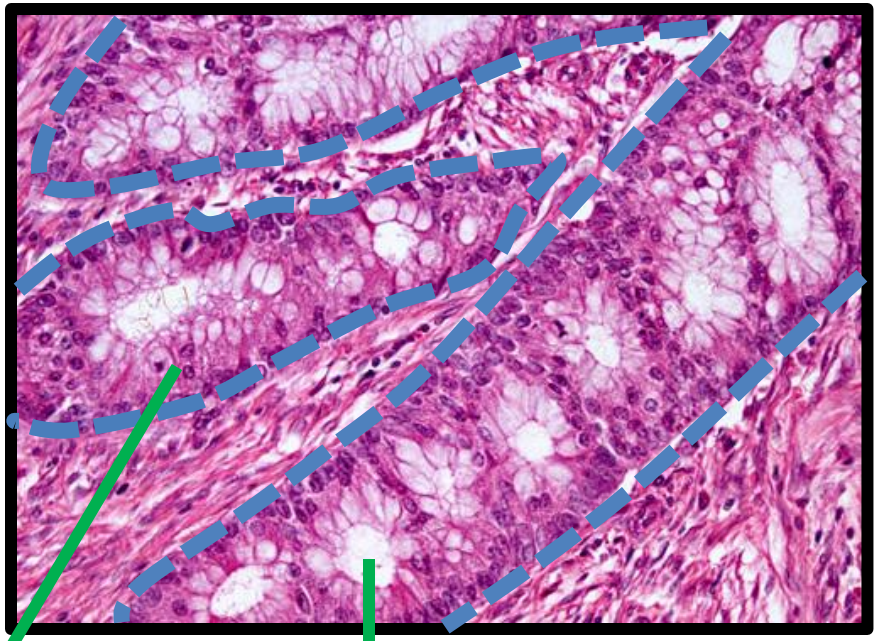
Clinical Questions:

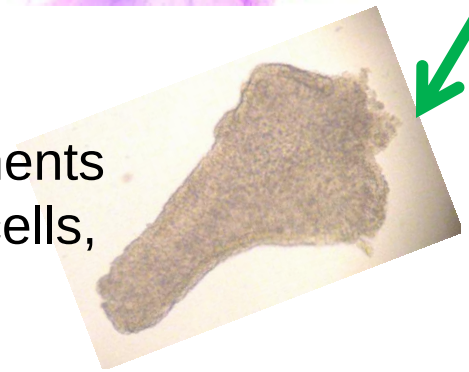
How Do We Prevent Cancers? Are Tumors “Born To Be Bad”?

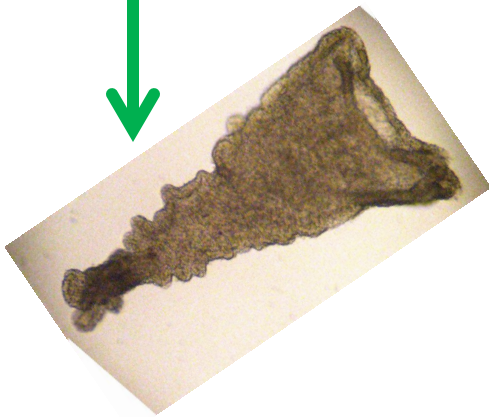


Colorectal Cancers Have Structure (Adenocarcinomas With Glands)



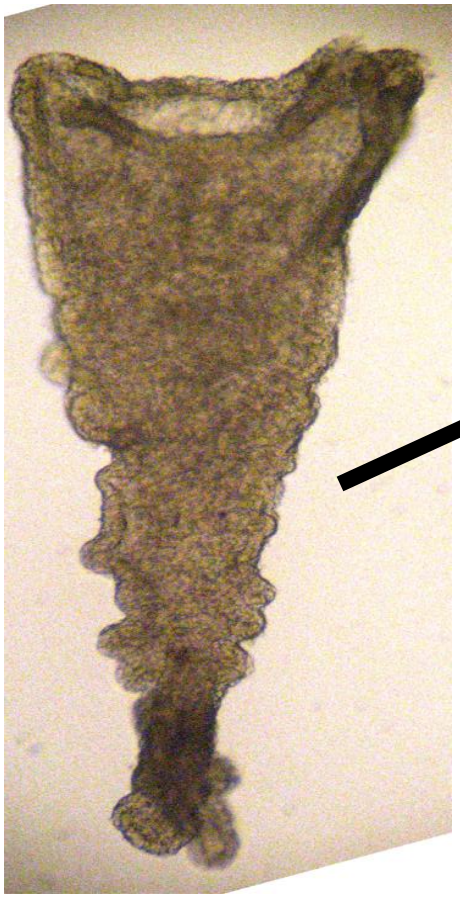






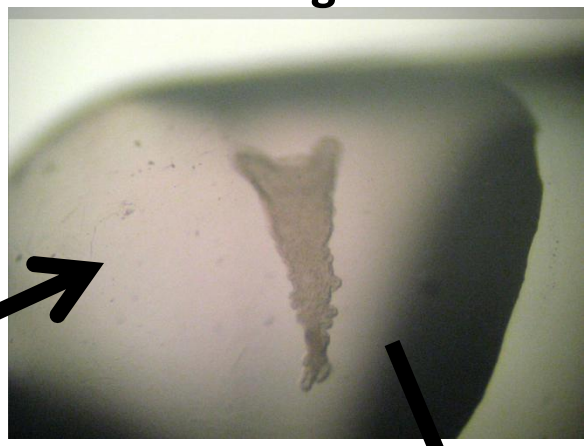
Tumor Gland Fragments
(~10,000 adjacent cells,
>95% pure)

Single Tumor Gland/Fragment Analysis (cell neighbor analysis)



~ 10,000 Adjacent Tumor Cells

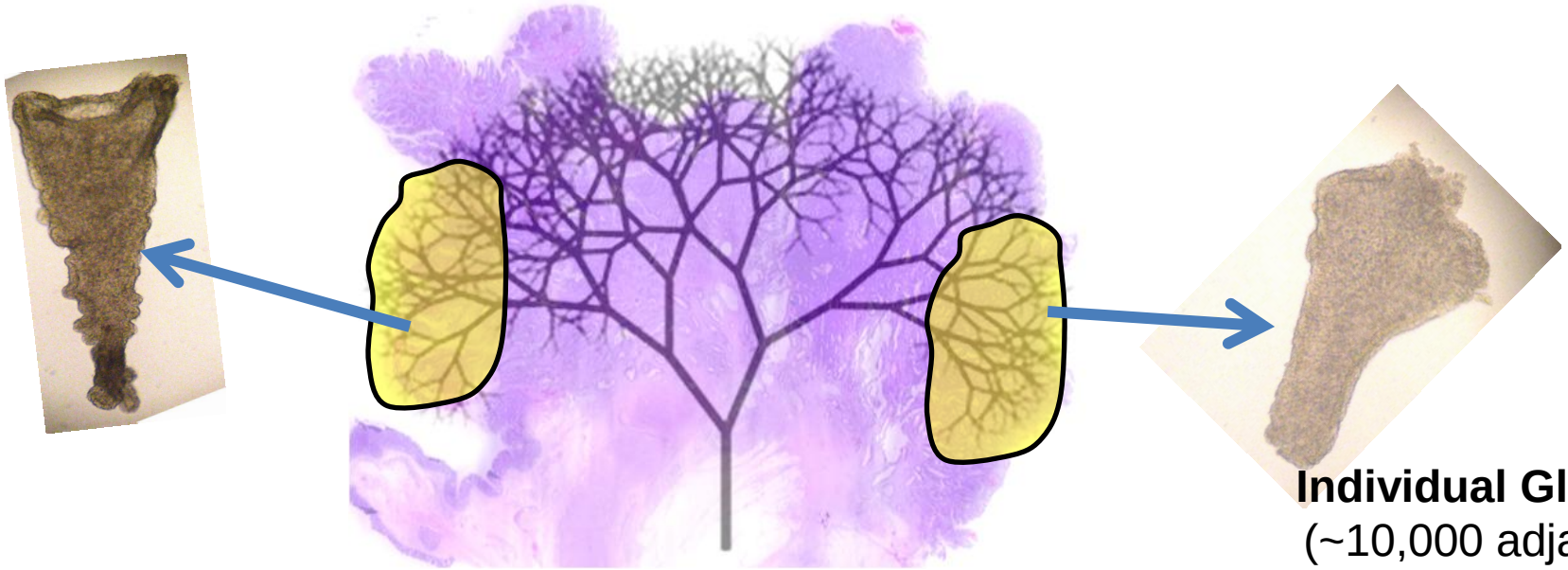
microfuge tube



1. Chromosome Copy Number Alterations (CNA, SNP-chips)
2. DNA Passenger Methylation Patterns (bisulfite sequencing)
3. Targeted Resequencing (AmpliSeq/IonTorrent)

Relative Error and Mitotic Rates ("molecular clocks")

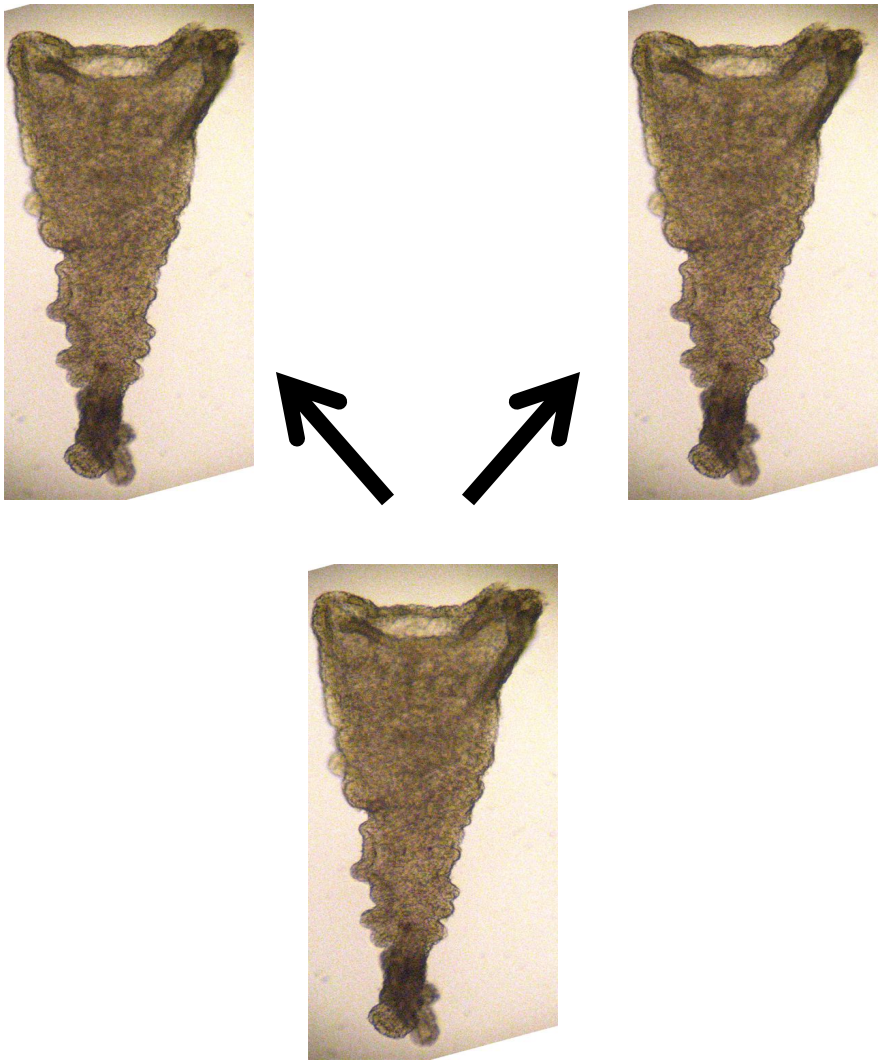
DNA base fidelity	~10^{-9} per base per division	
DNA methylation	~10^{-5} per base per division	Stepwise Changes
Chromosome CNA	~10^{-2} to 10^{-4} per division	



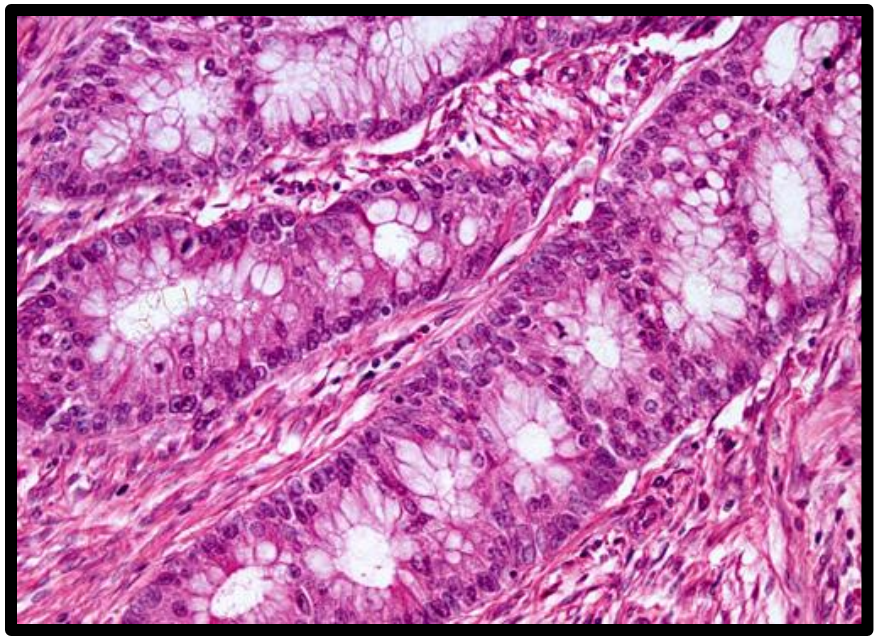
Individual Glands
(~10,000 adjacent cells)

Tumor Growth:

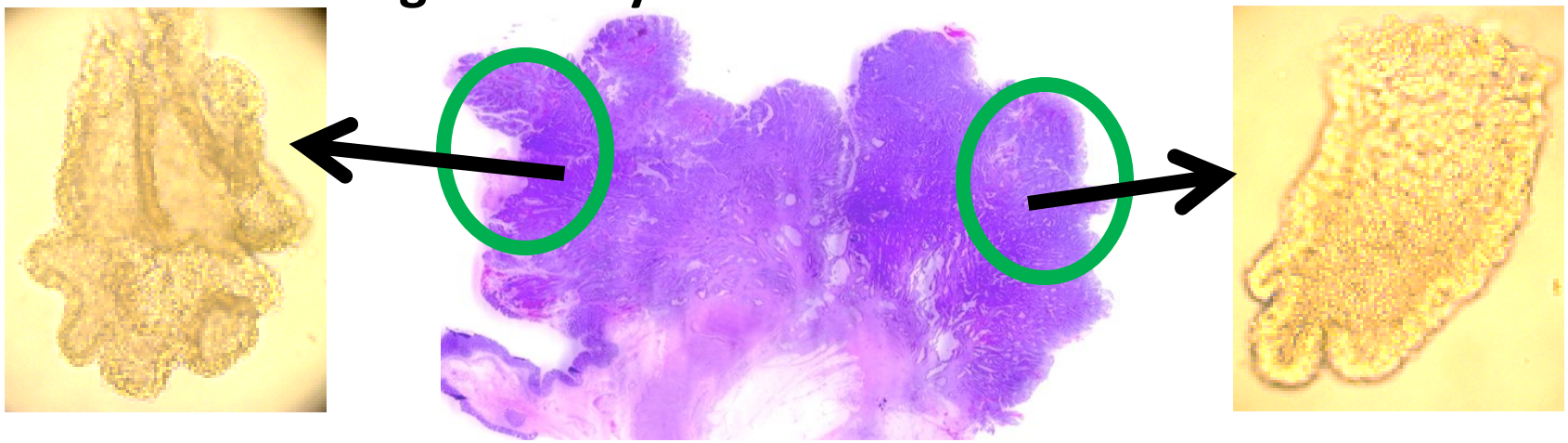
1) **Cells Divide**
2) **But Growth is Through Gland Division (Fission)**



Therefore Glands May Be Stable Physical Structures: Glands Can "Age" (their cells become polymorphic)



Experimental Strategy: Sample Multiple Tumor Glands DNA Passenger Methylation Patterns



six cancer glands

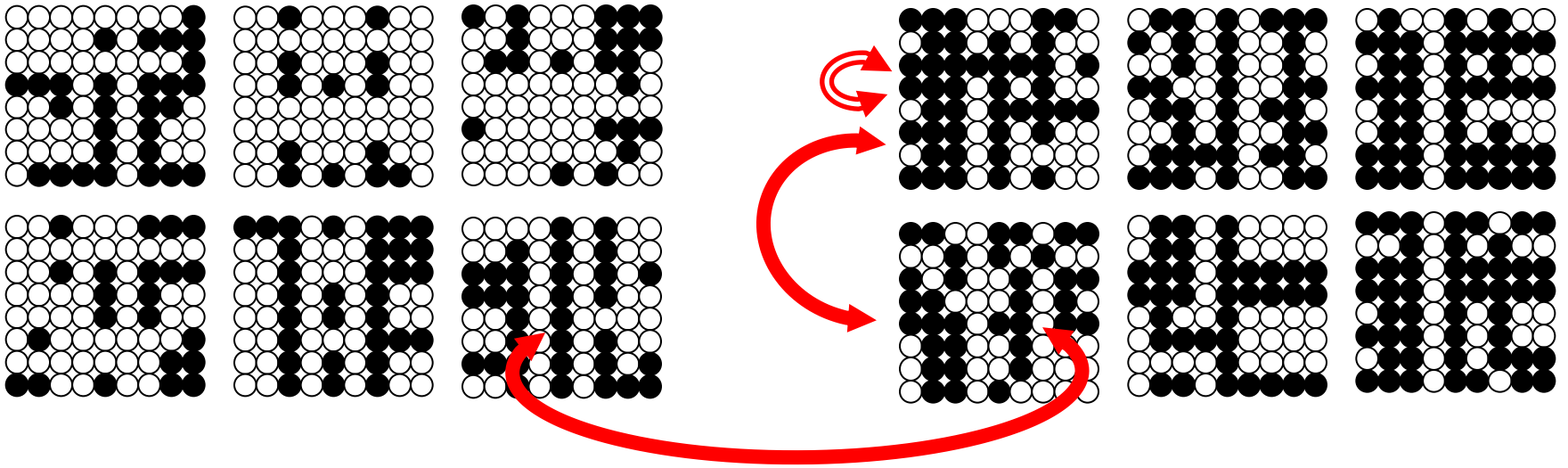
left side

5’ 3’ 5’ 3’ 5’ 3’

six cancer glands

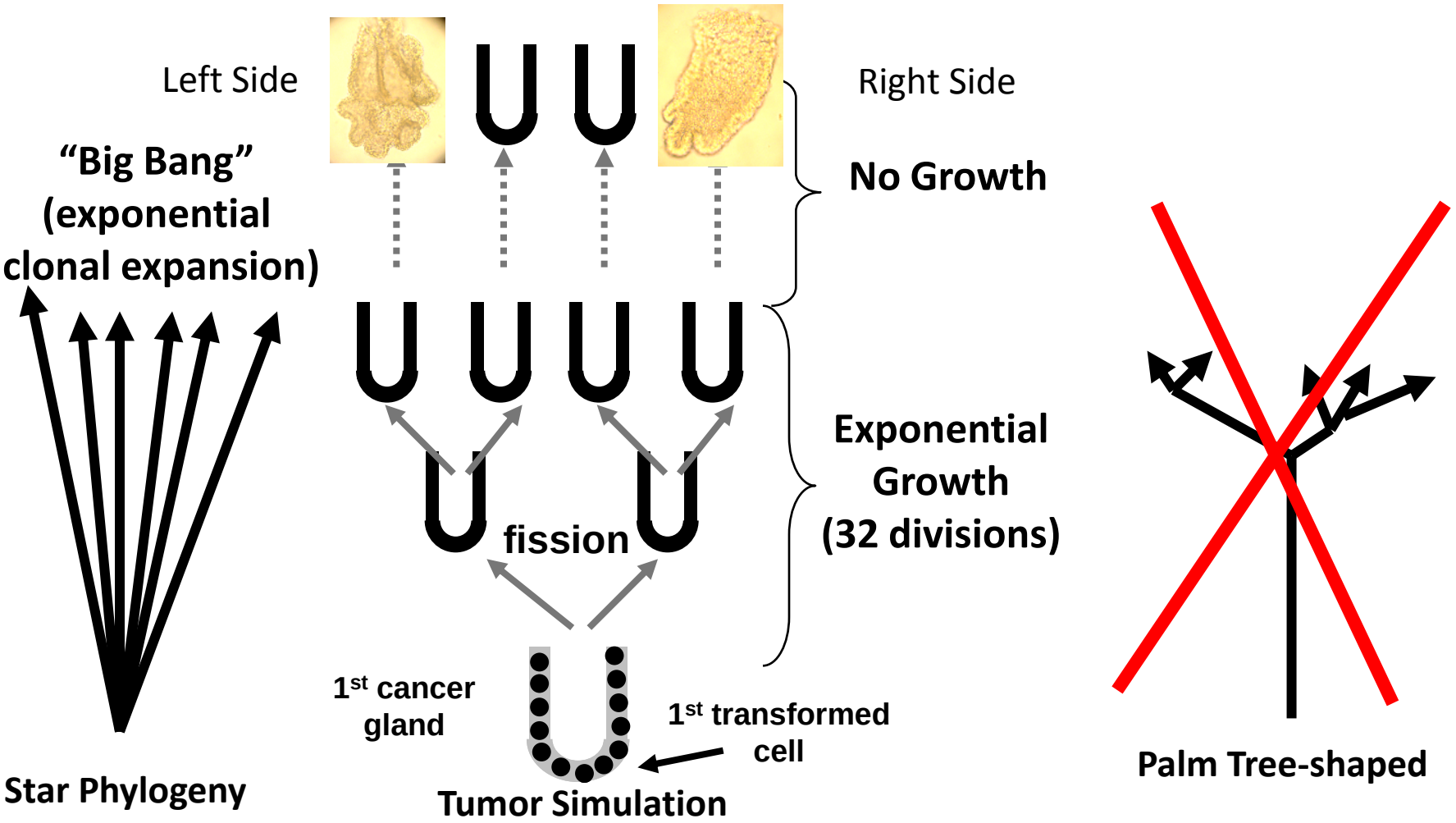
right side

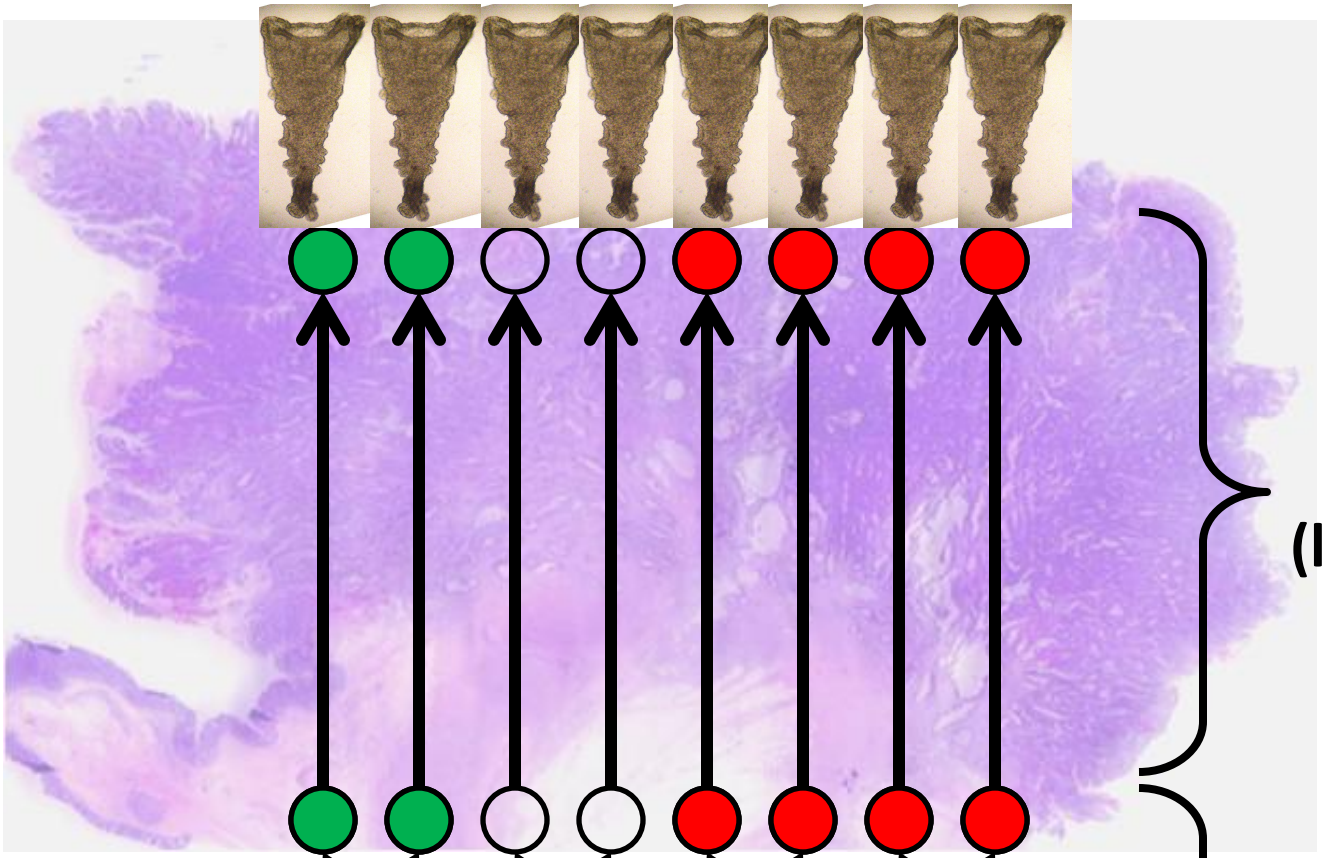
5’ 3’ 5’ 3’ 5’ 3’



Passenger DNA Tumor Gland Methylation:
More Consistent With A Star Phylogeny (single clonal expansion)

1. Gland Are "Old" or Diverse Populations (Stable)
2. Individual Glands Are Almost As Old or Diverse As Their Tumors
3. No Evidence of New or Old Parts (Equally Old or Young)





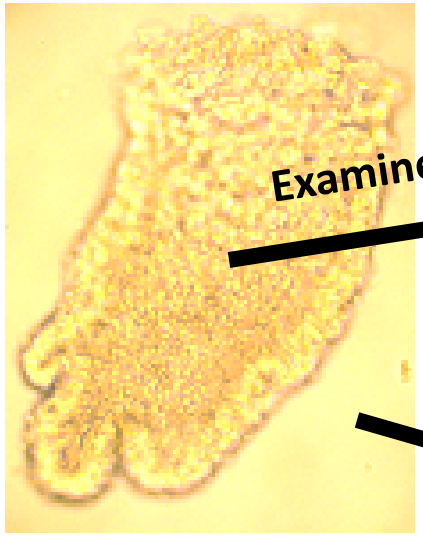
"Aging"
(long time)

Growth
(short time)

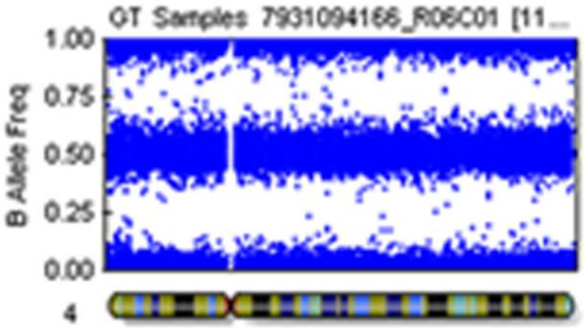
Tumor Star Phylogeny:

Inherent Non-Chaotic
Mechanism To Generate Variation (ITH)

Chromosome CNAs
(Chromosomal Instability (CIN))



Examine Single Gland



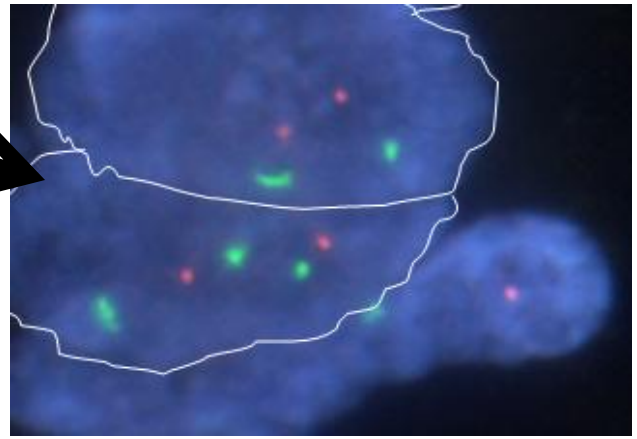
2N
Tumor Gland
"Quantum Mechanics"

SNP Microarray:

<u>Average</u> of gland = "Integer Value"

Examine Single Cells

Gland



Stepwise Chromosomal Changes:
Gains <u>and</u> Losses

FISH: CIN PRESENT
(different ploidy)

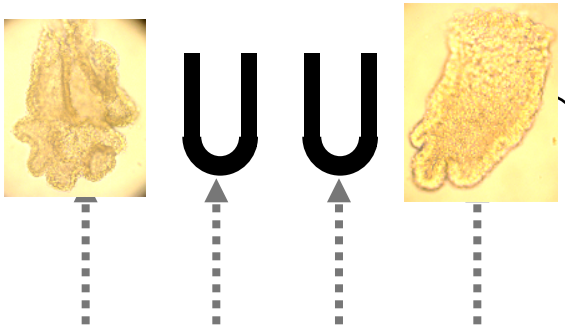
Why Are Tumor Glands "Quantum"?

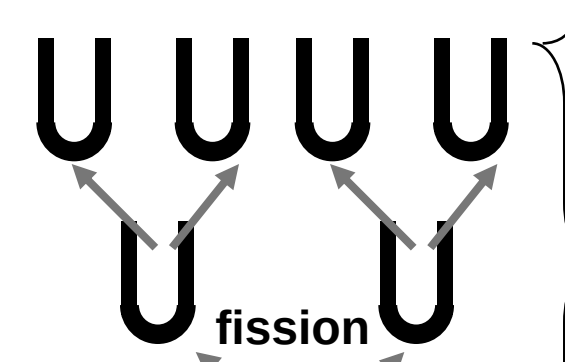
Model:

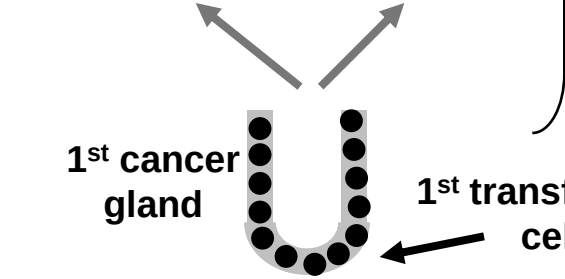
1) Single Cell Start is Quantum (CN is integer, 0,1,2,3,4.....)

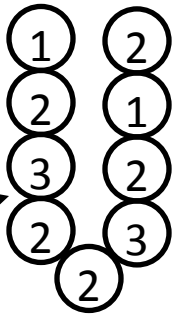
2) Glands Are Formed Soon After The Start And Therefore Tend To Be "Quantum"

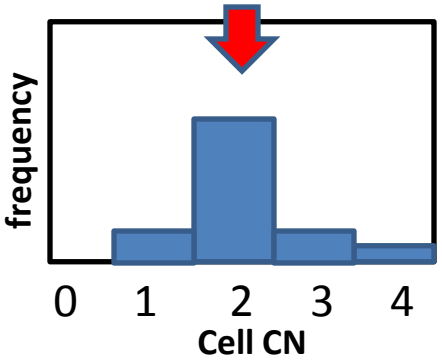
Average gland CN = "start"

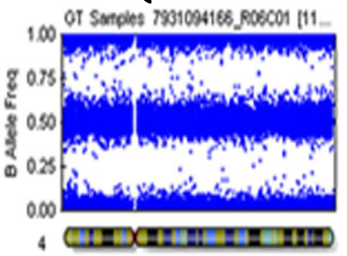




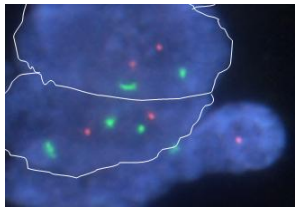




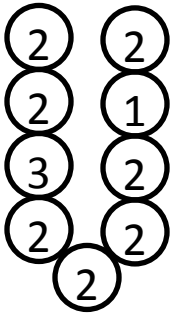


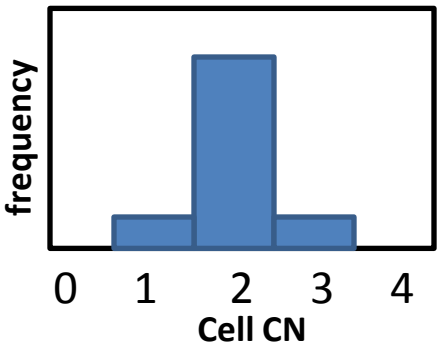


Gland CN

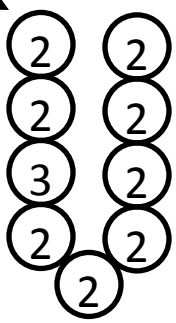


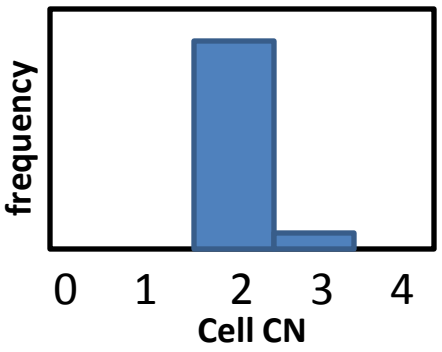
No Growth





Exponential Growth (32 divisions)





fission

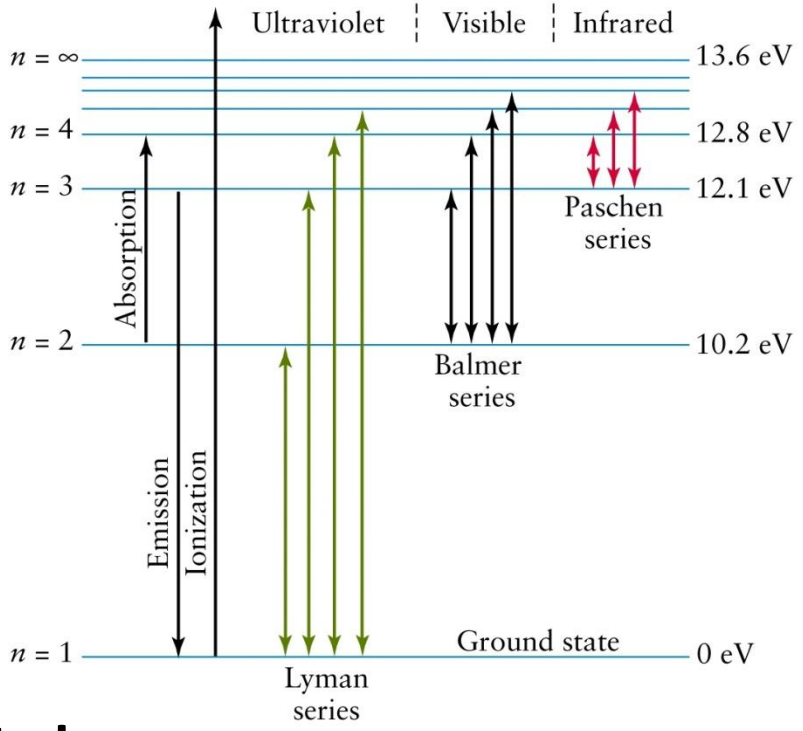
1st cancer gland

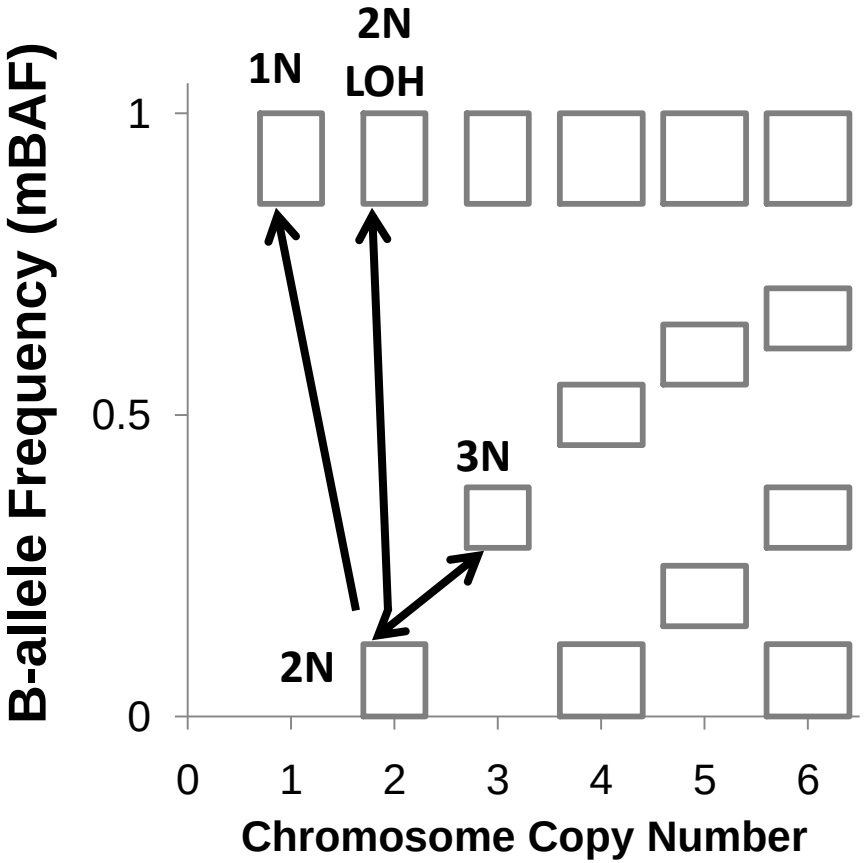
1st transformed cell

2N

Tumor Simulation

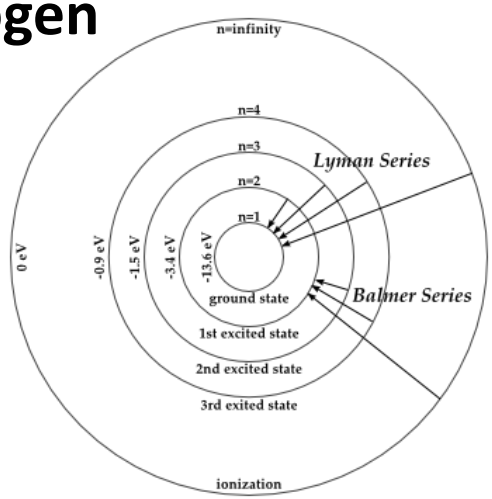
"Quantum Mechanics": Certain "States" Are Favored





Cells Are Inherently Quantum

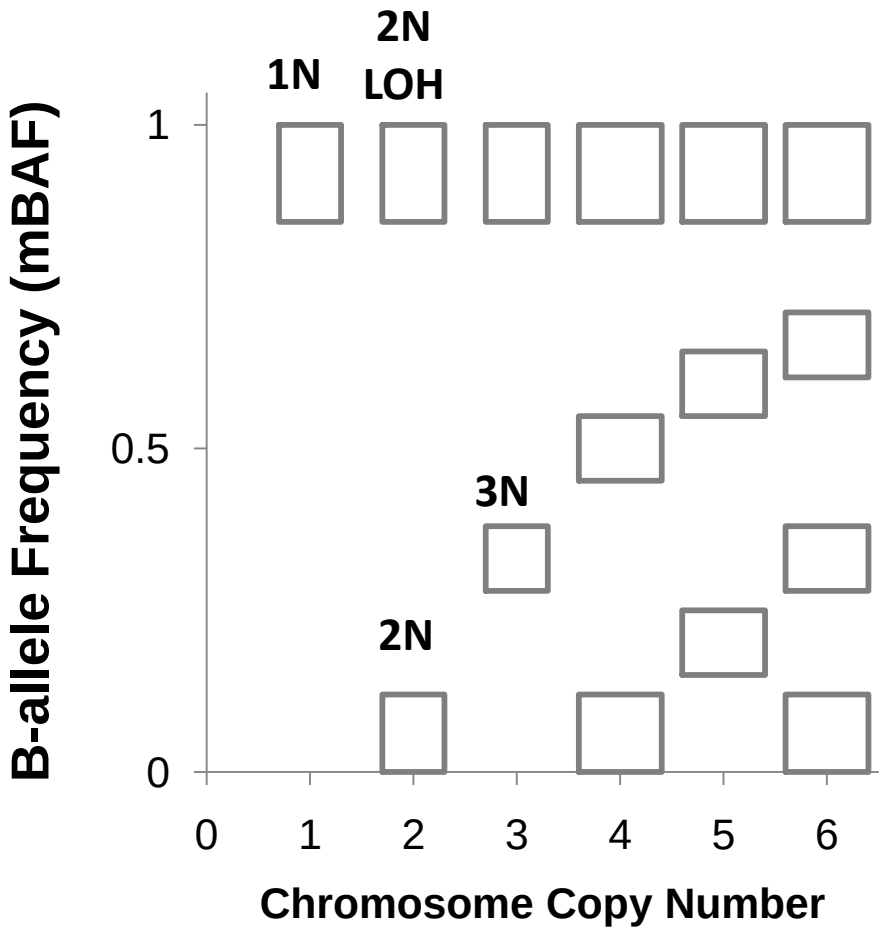
Hydrogen Atom



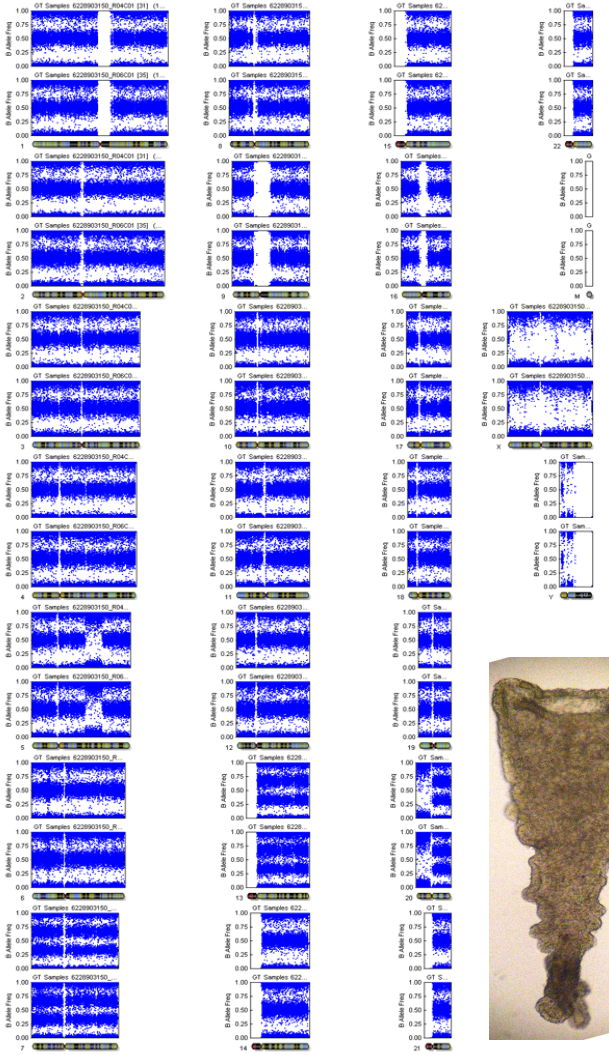
Glands Are Mixtures of Cells

Closely Related

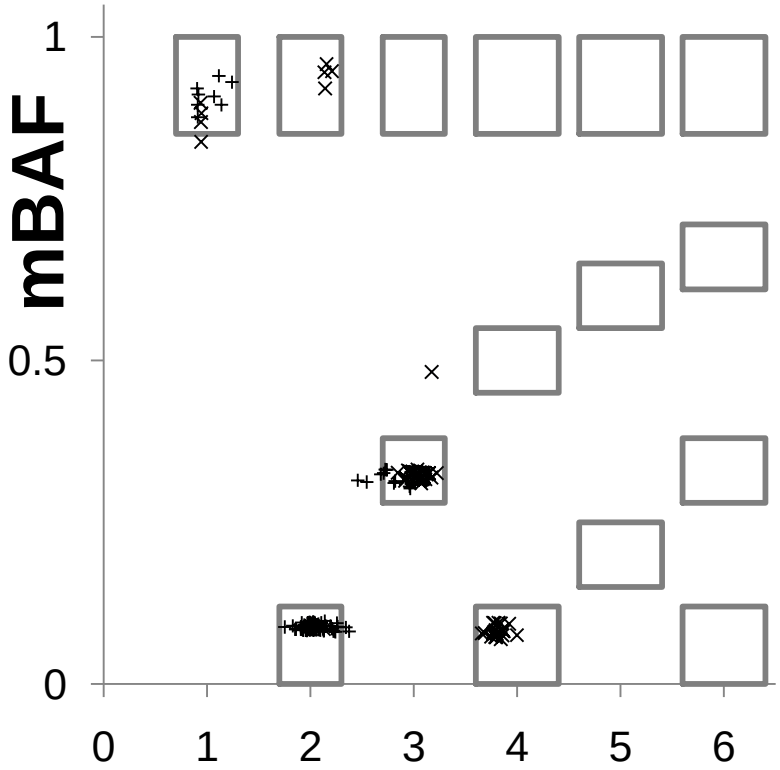
Visualizing Gland Chromosome Ploidy
("quantum integer normal values")



SNP microarrays

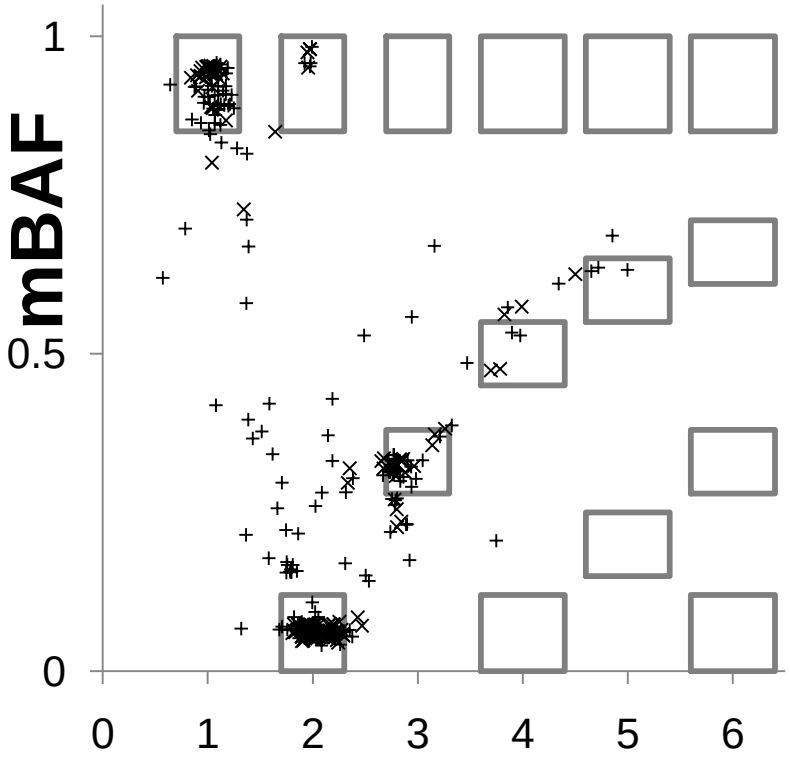


Despite "CIN" Most Gland Chromosome Fragments Are "Fixed" (near "quantum" or integer values)



Adenoma

(8 glands)

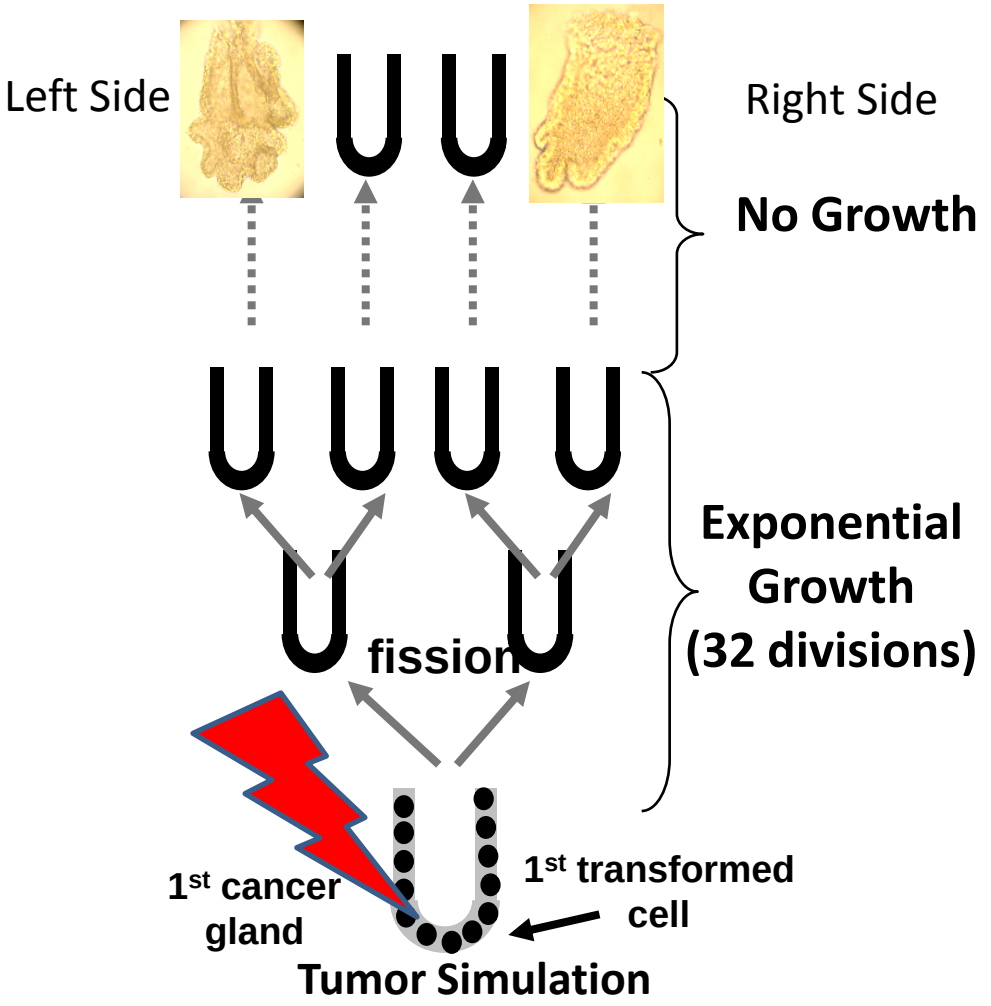


Cancer

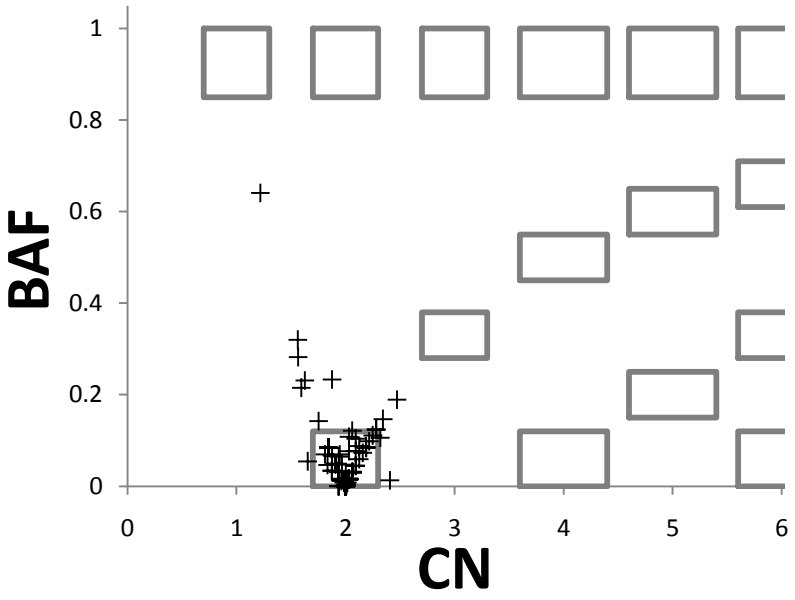
(8 glands)

Tumor Simulations of CIN

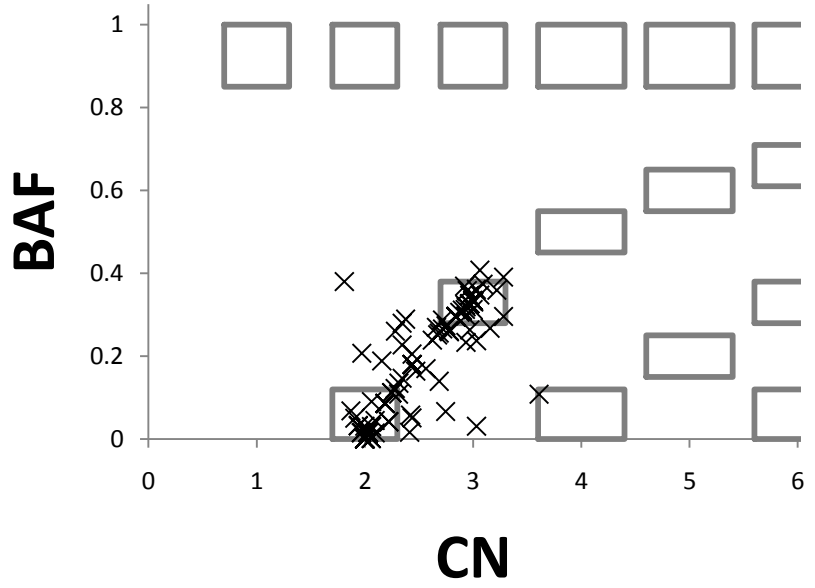
Start: 2 chromosomes,
200 divisions



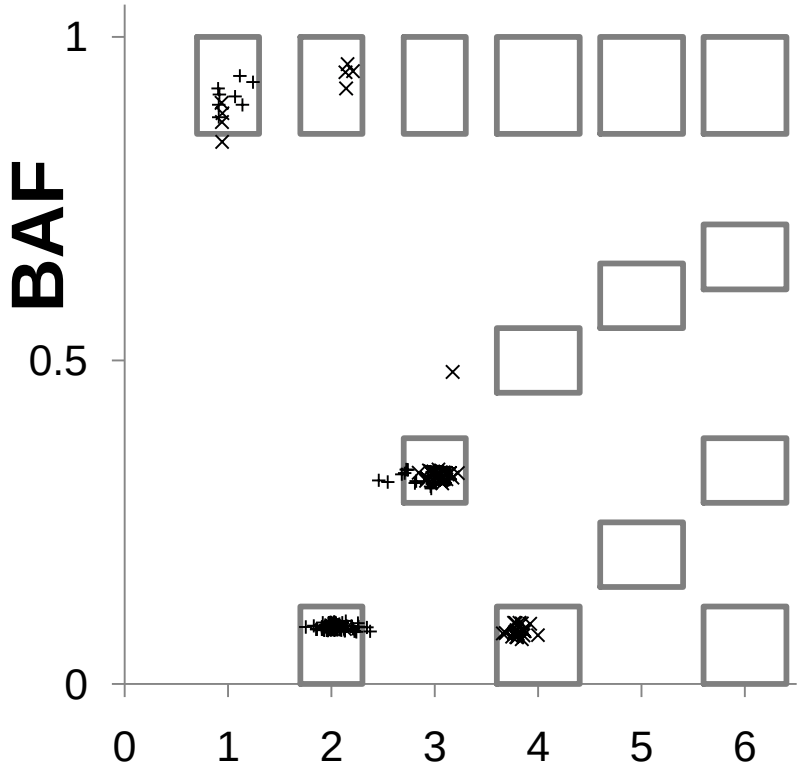
Random CNA



Force CNA (+1) At First Division

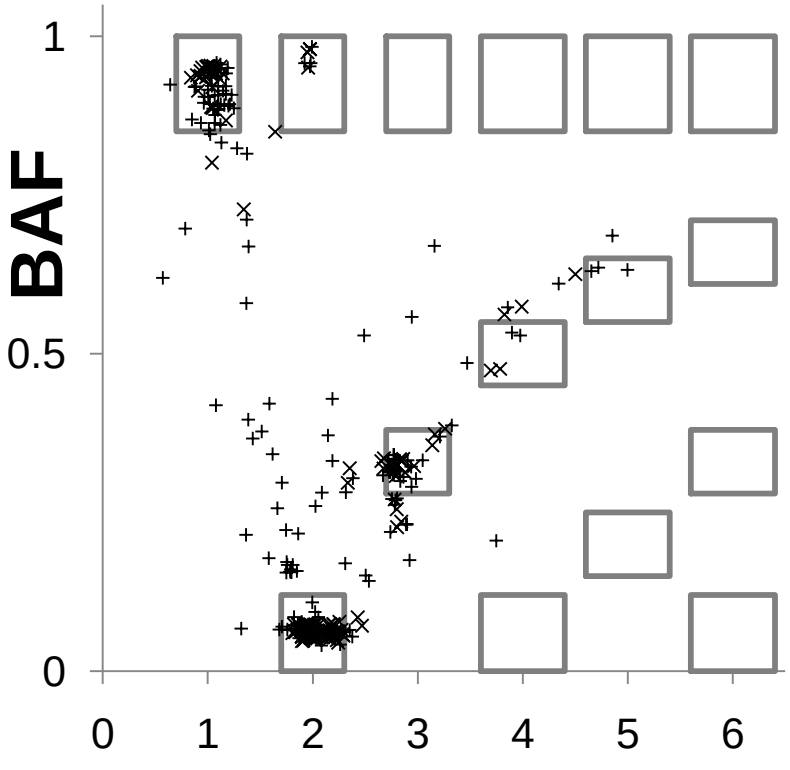


Despite "CIN" Most Gland Chromosome Fragments Are "Fixed" (near "quantum" or integer values)



Adenoma

(8 glands)

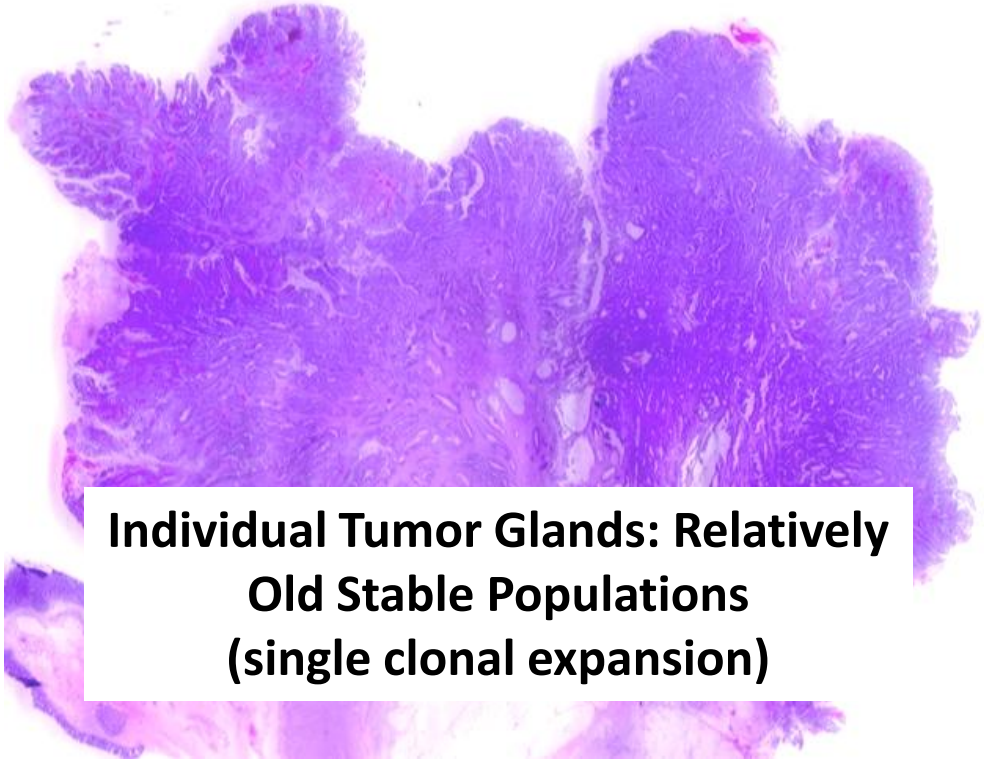


Cancer

(8 glands)

Summary of Tumor Gland Alterations

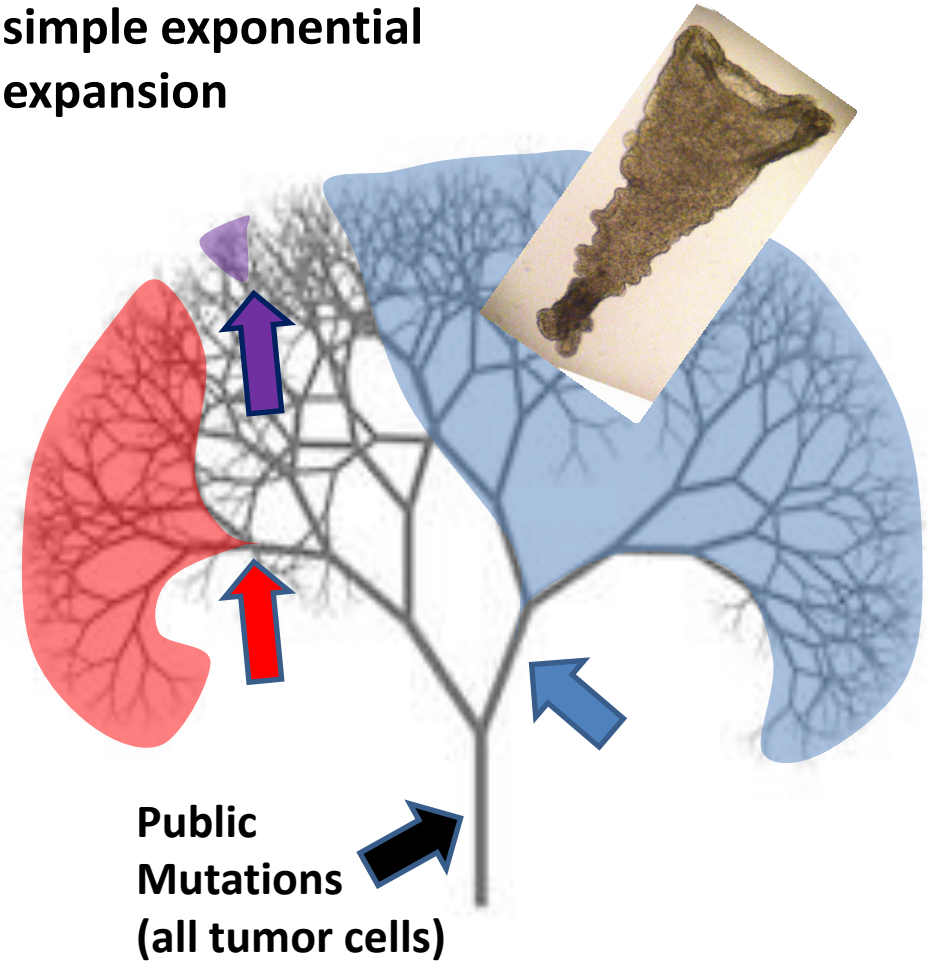
1) Passenger Methylation Patterns: Diverse
2) FISH Chromosome CNAs: Diverse
3) SNP Microarray: Many Average Gland CNAs Are "Quantum" (reflect CN of the first tumor cells)



Individual Tumor Glands: Relatively Old Stable Populations (single clonal expansion)

What About Point Mutations?

simple exponential expansion



Whole Tumor

Public: 100% cells
Private:
Division 1: 50%
Division 2: 25%
Division 3: 12.5%
Division 4: 6.25%
Division 5: 3%

Single Gland

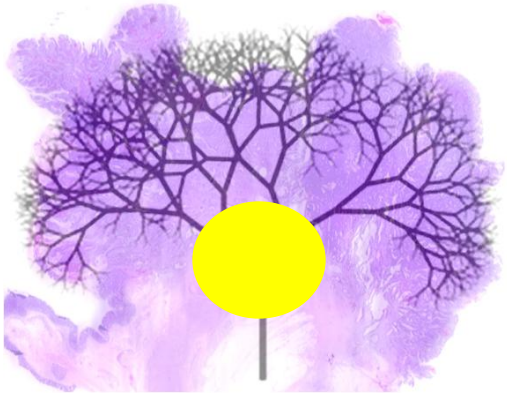
Public: 100% cells
Private:

0 to 100%

Possible Gland Point Mutation Frequencies

1) Infinite Possible Values (0 to 100%)
----Genomic Instability
----Migration and Mixing

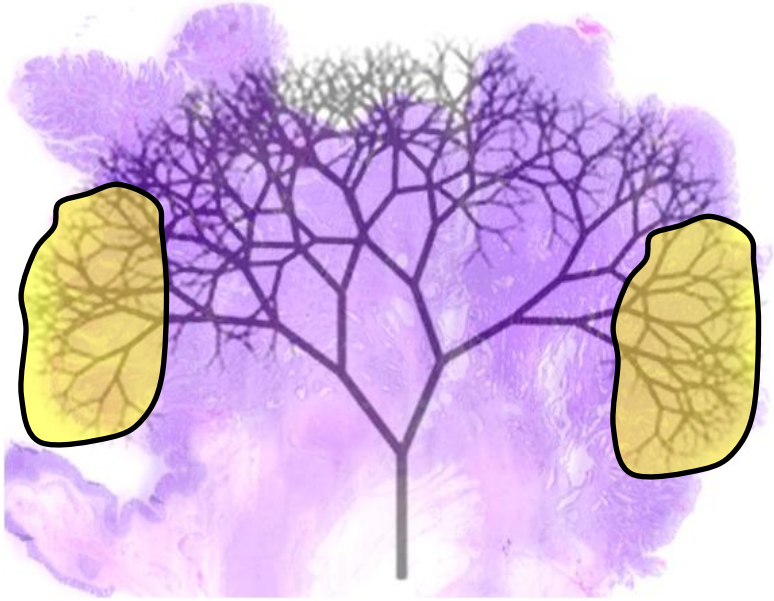
2) "Quantum" Values (1N, 2N, 3N…….)
----<u>Detectable</u> Mutations Are Public
and Early Private Mutations
----Individual Glands Are Old, Stable Populations
(fixation or lost)

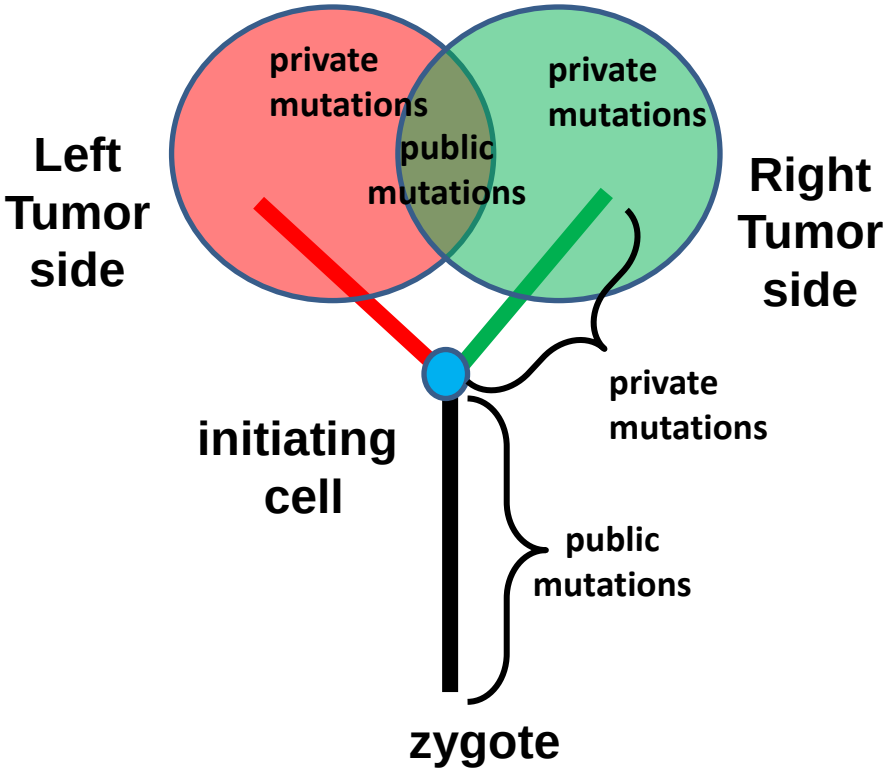


(Hint: Gland Chromosome CN and Detectable Point Mutation Frequencies Are Entangled)

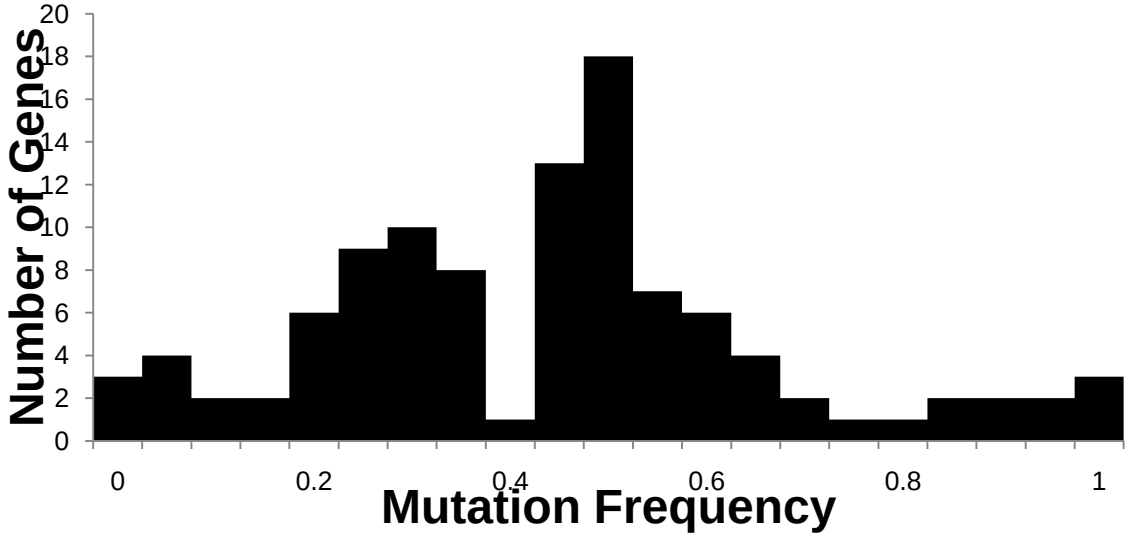
Experimental Approach

1) Bulk Sample Opposite Tumor Sides
2) NGS (Illumina, Exome Sequence, 50X)
3) Identify Public and Private Point Mutations (MuTec, Somatic Sniper)
4) Resequence Mutations In Bulk Sample and Individual Glands (AmpliSeq, IonTorrent ~100X+ coverage)

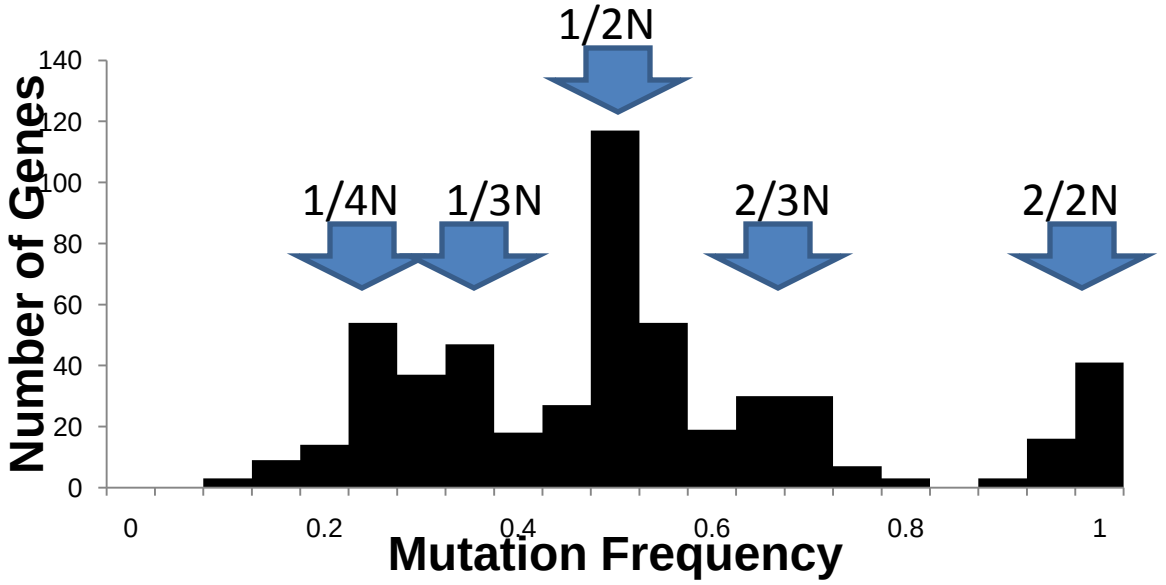




Bulk Resequencing Data: Continuous Mutation Frequencies



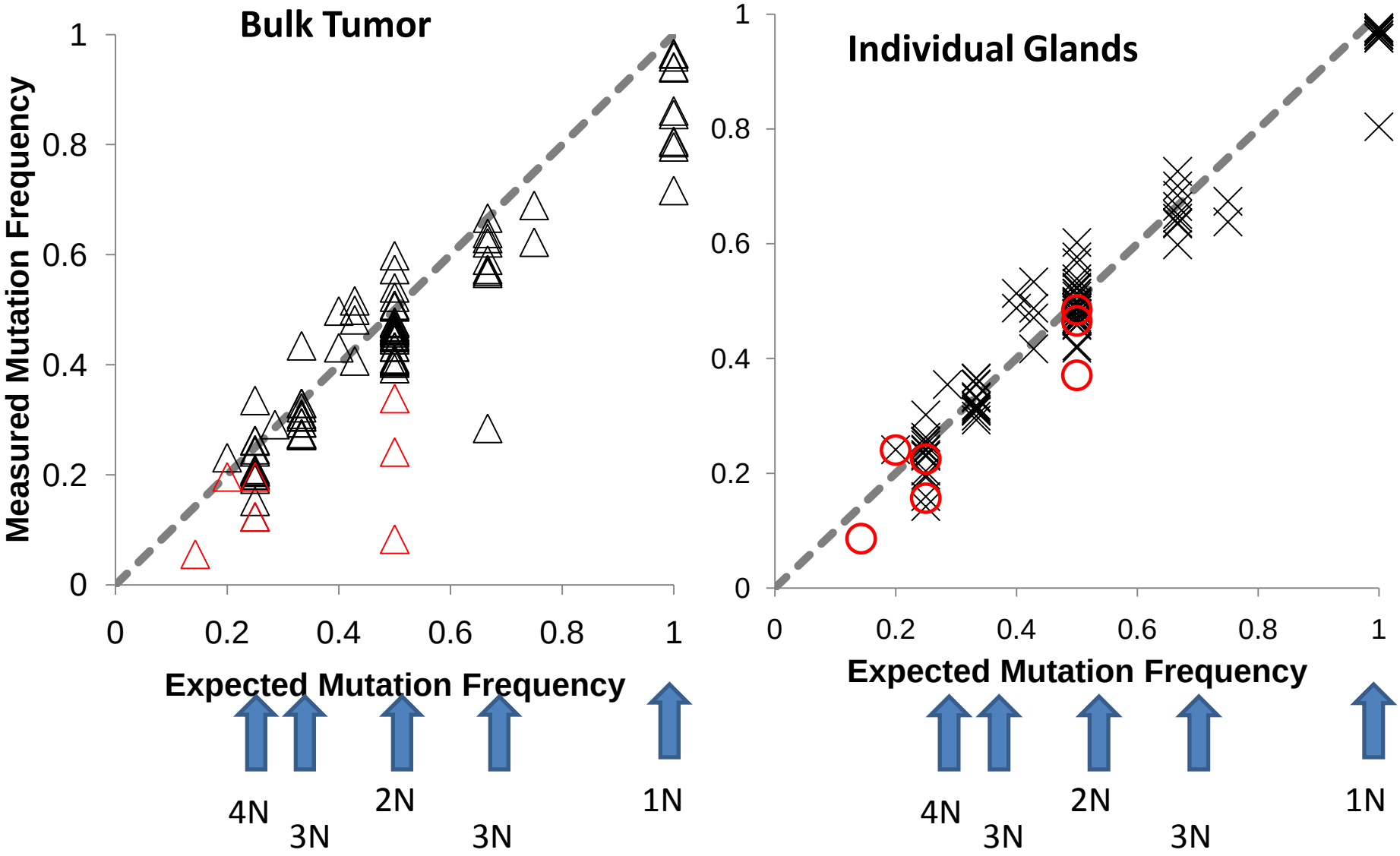
Gland Resequencing Data: "Quantum" Mutation Frequencies



Mutation Frequency With Respect To Ploidy

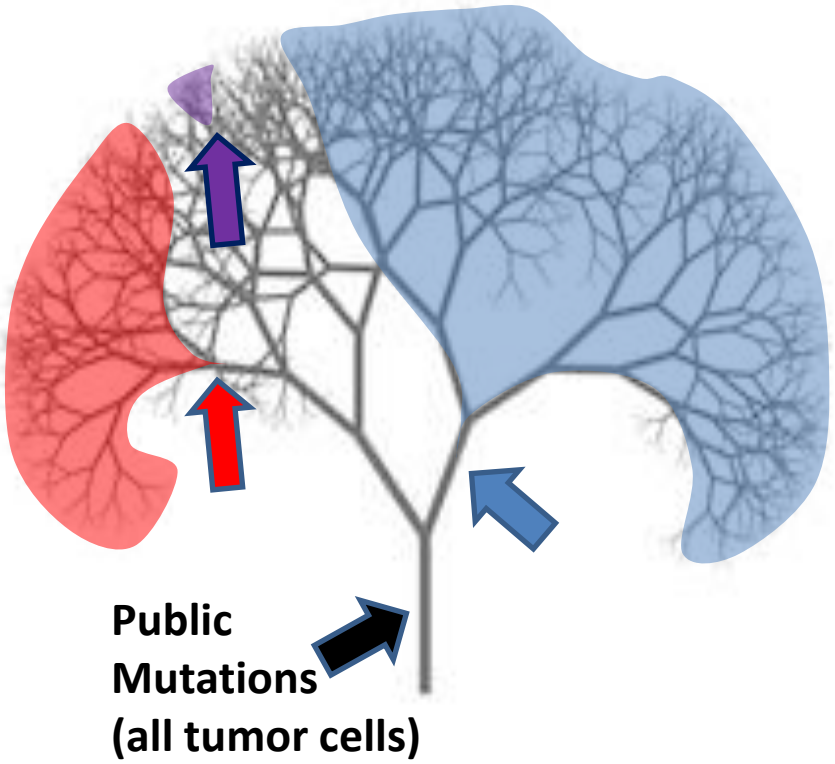
BLACK Symbols = Public Mutations

RED Symbols =Private Mutations

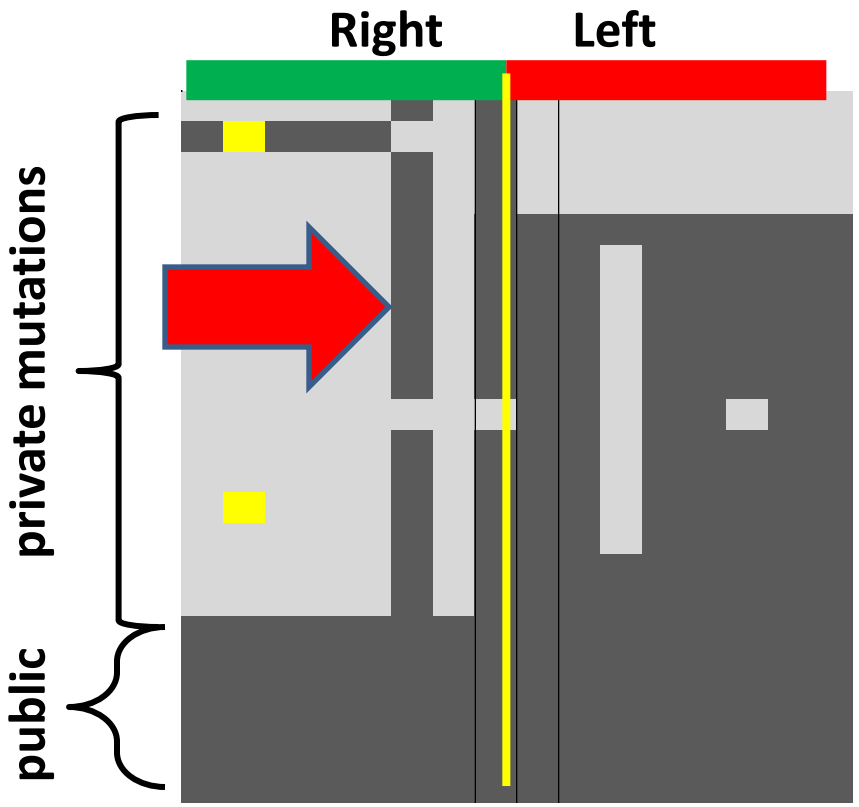


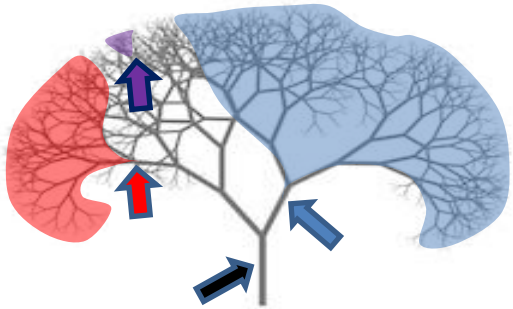
Summary of Tumor Gland Alterations

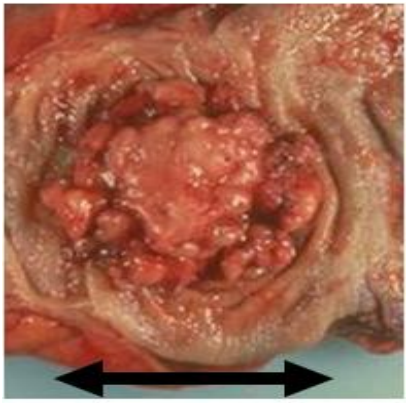
1) Passenger Methylation Patterns: Diverse
2) FISH Chromosome CNAs: Diverse
3) SNP Microarray: Many Average Gland CNAs Are “Quantum”
4) Mutation Resequencing: “Quantum” or “Fixed” Detectable Point Mutation Frequencies



How Did A Gland Cross To The Other Tumor Side?

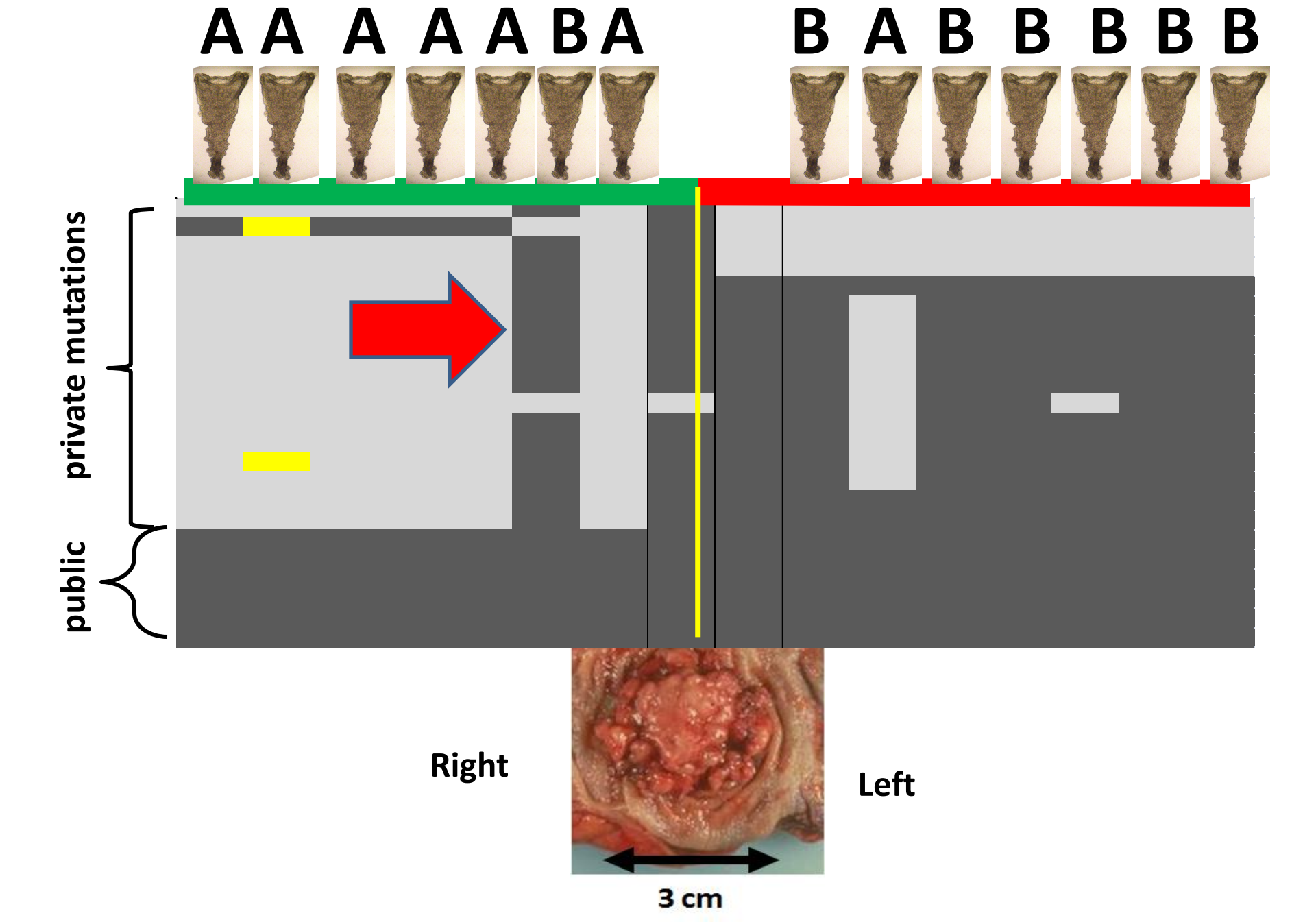




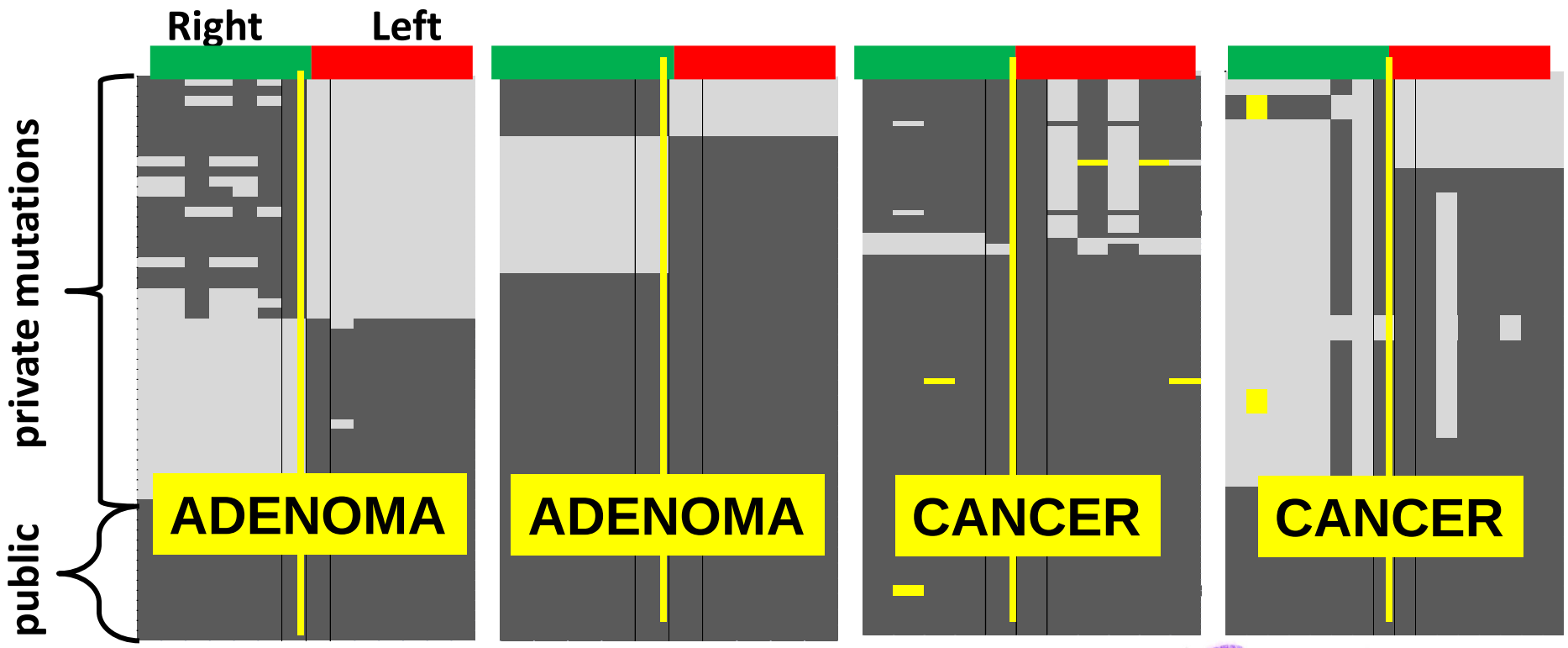


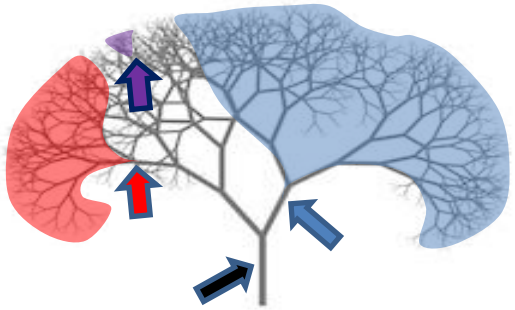
Cells Migrate But
Glands Don't Migrate Much

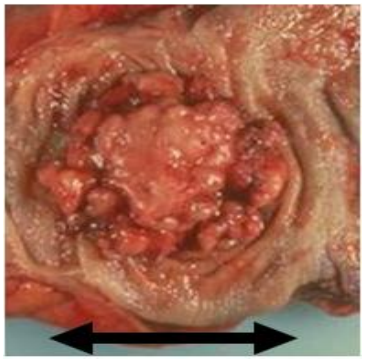
How Did A Gland Cross To The Other Tumor Side?

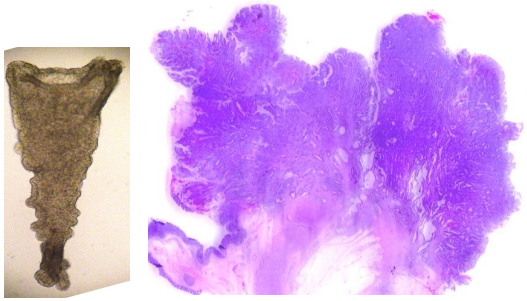


How Does A Private Mutation End Up On Both Sides Of A Tumor?









Cells Migrate But
Glands Don't Migrate Much

"Born To Be Bad"

What is "Bad" Clinically?: Death

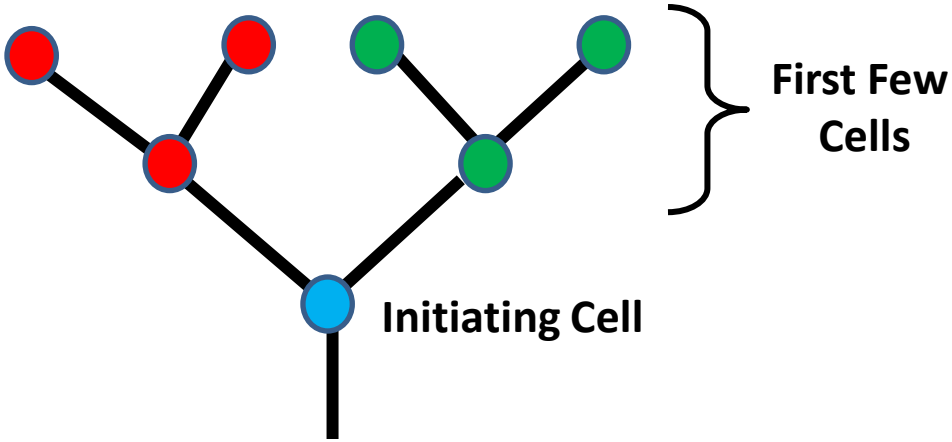
How Do Tumors Kill?

1) **Invasion**
2) **Metastasis**

Common Requirement of Invasion and Metastasis: <u>Abnormal Cell Mobility</u>

Late Event

Do The First Few Tumor Cells Move/Mix?

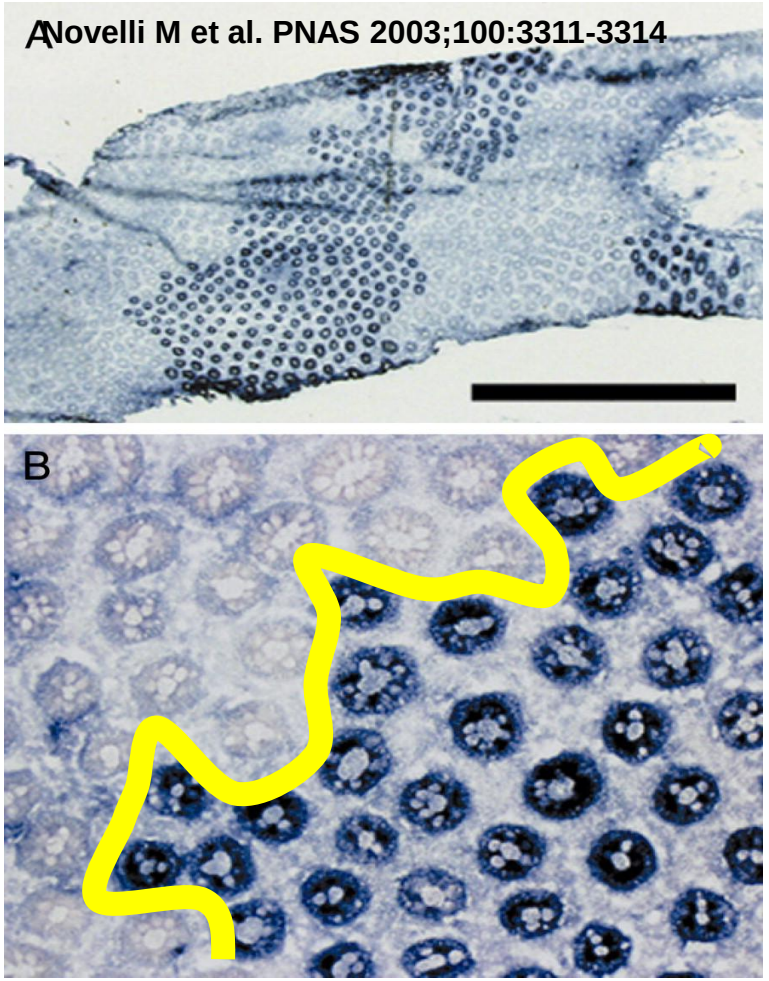


"Born To Be Good"

Cell Proliferation And Movement Is Normal
But Cell Intermixing Is Abnormal

Development:

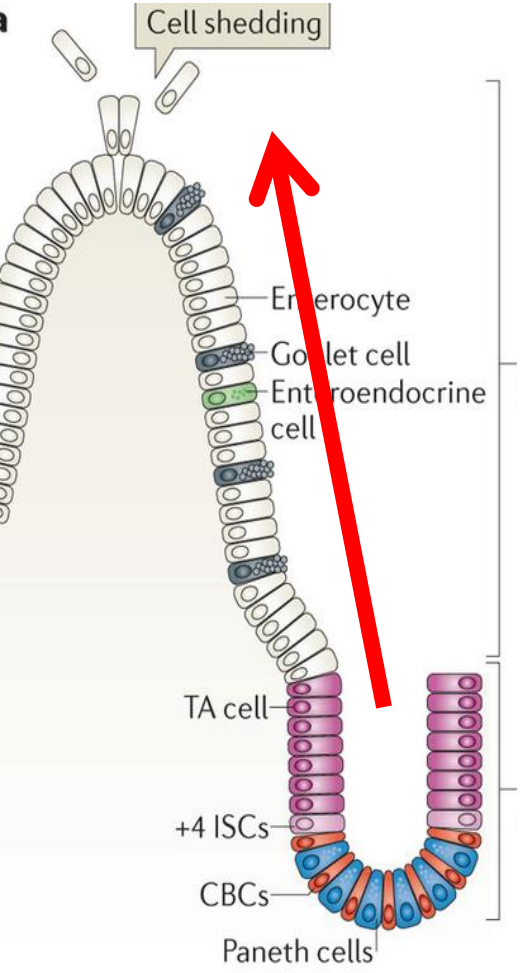
Clonal Patches

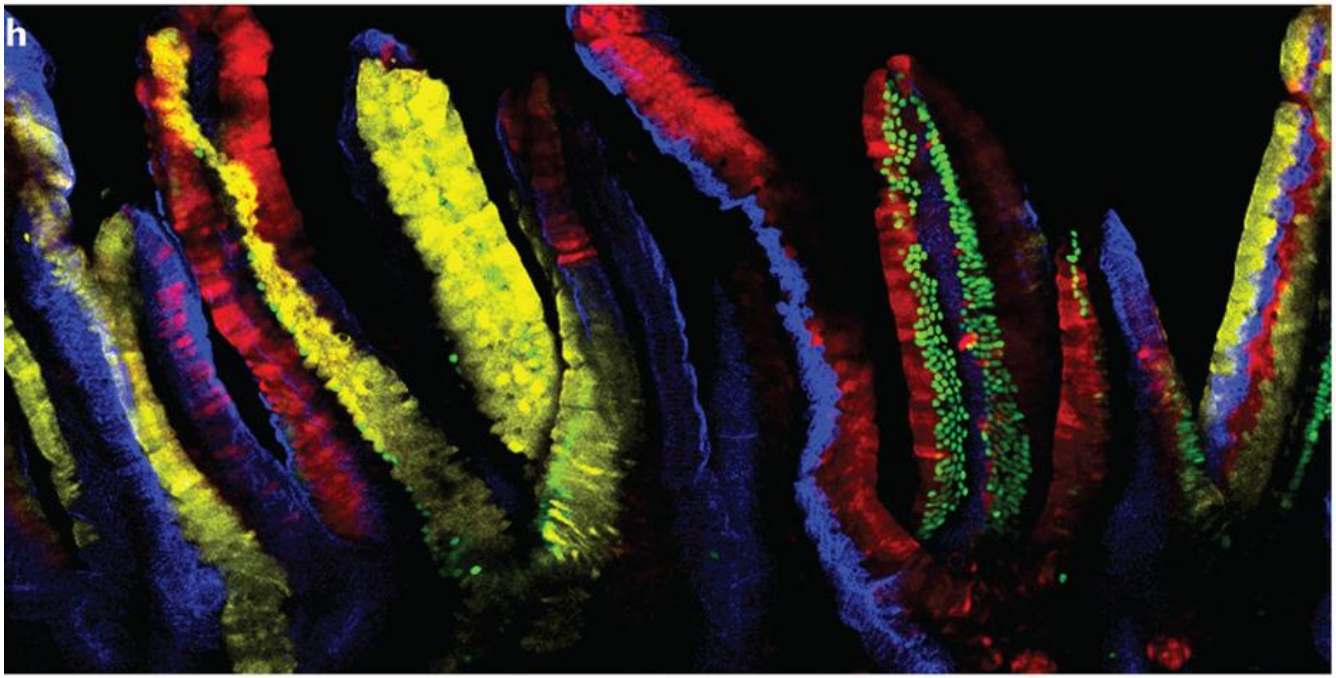


G6PDH expression: X-linked inactivation during human development

“Born To Be Good”

Cell Proliferation And Movement Is Normal
But Cell Intermixing Is Abnormal

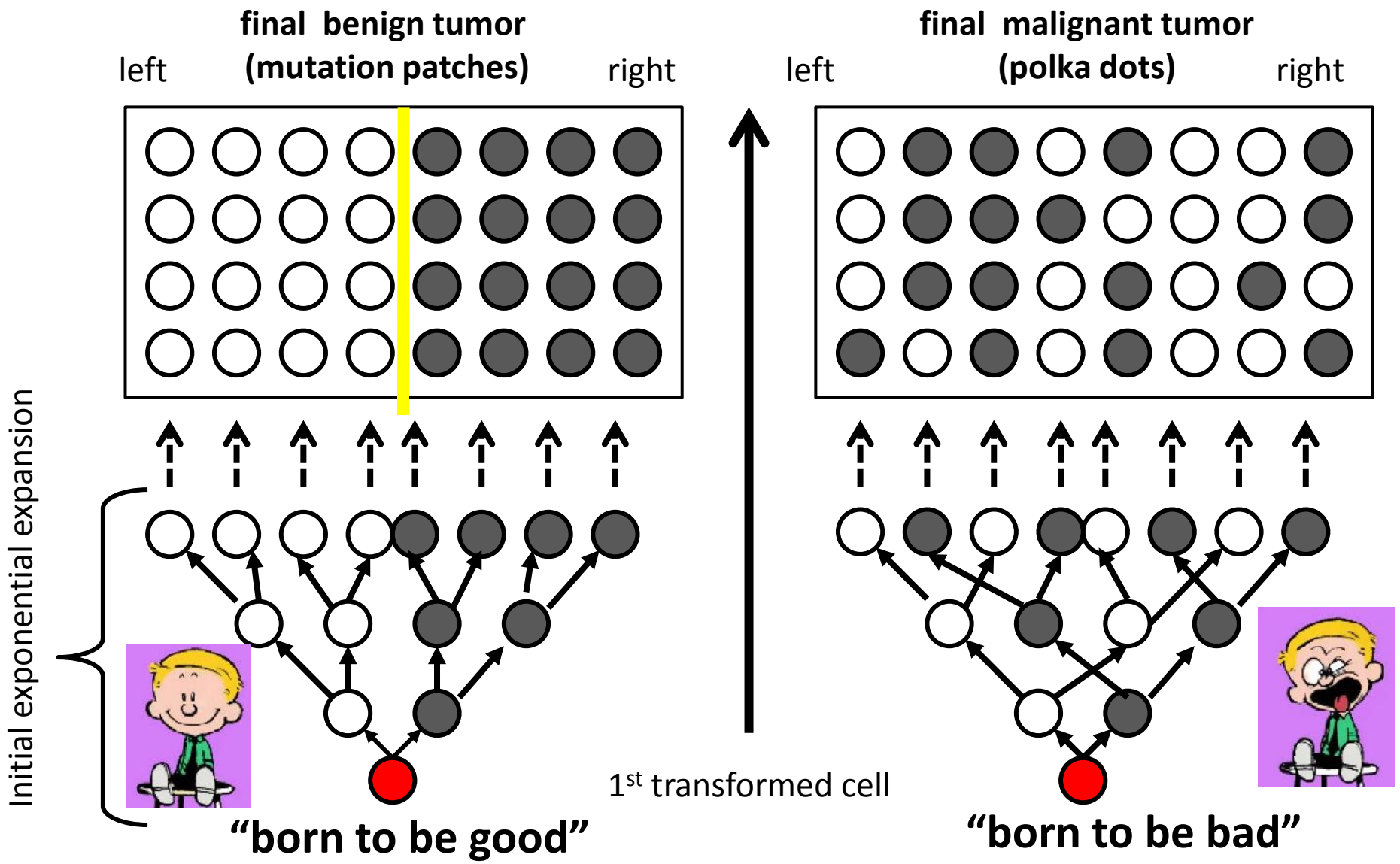




Intestinal Crypts:
Cell Migration in Orderly Columns

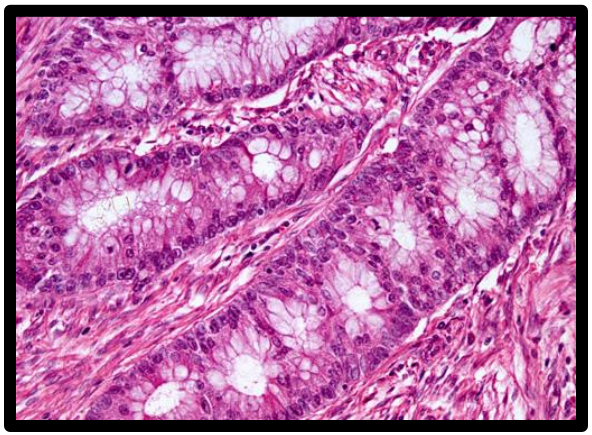
Born To Be Good/Bad

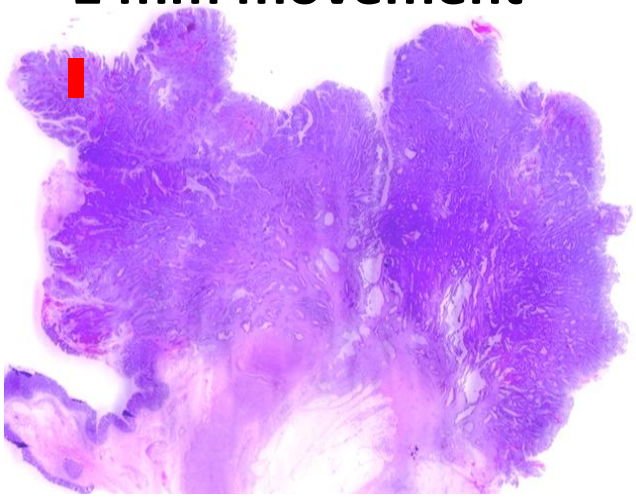
Effects of Early Cell Mixing



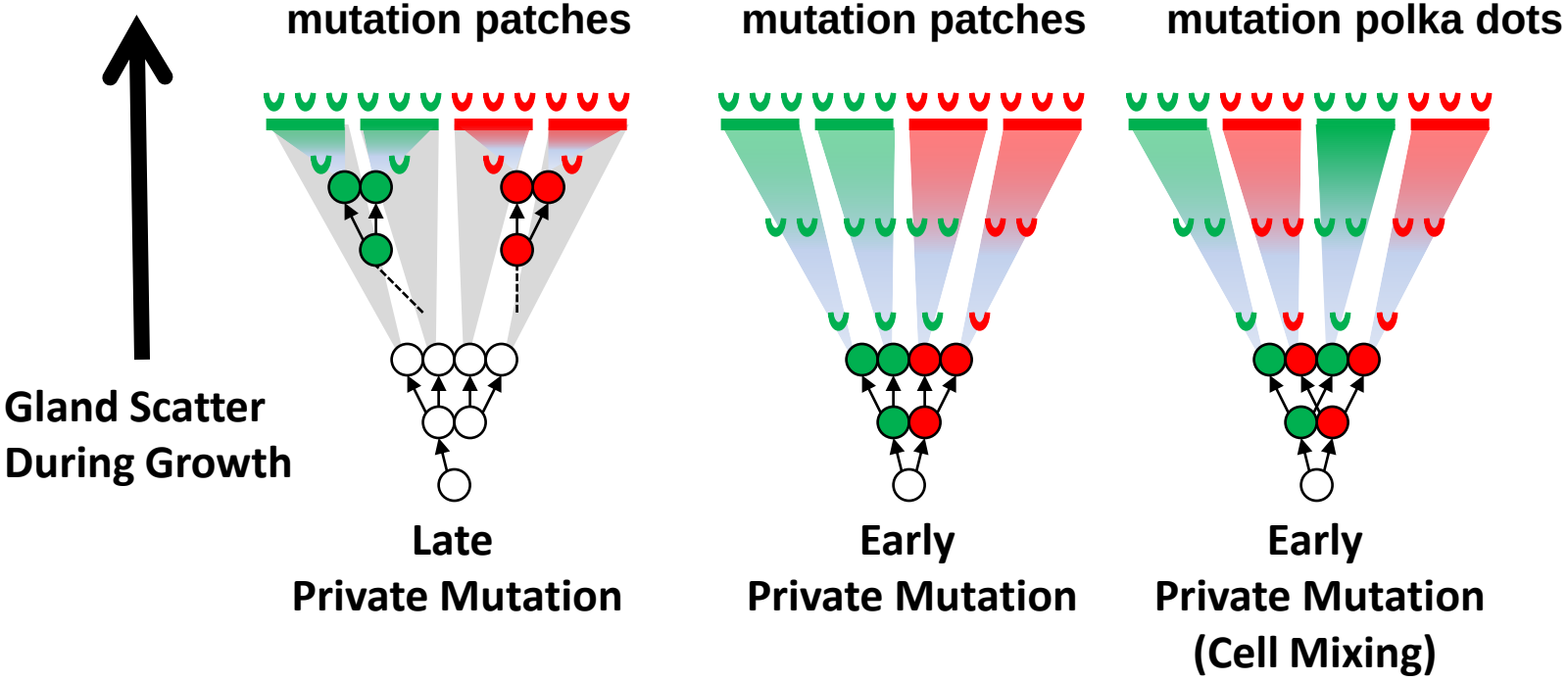
1 mm movement

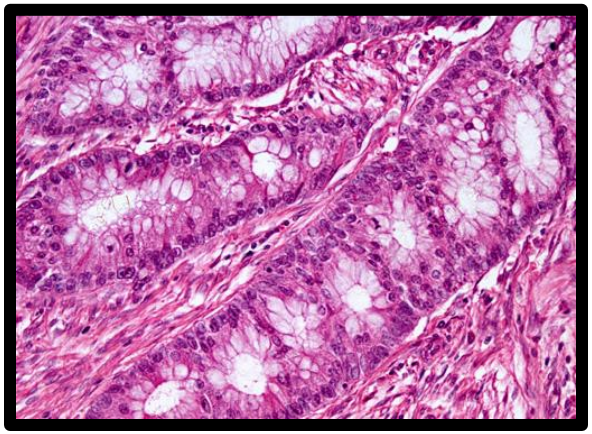
1 mm movement





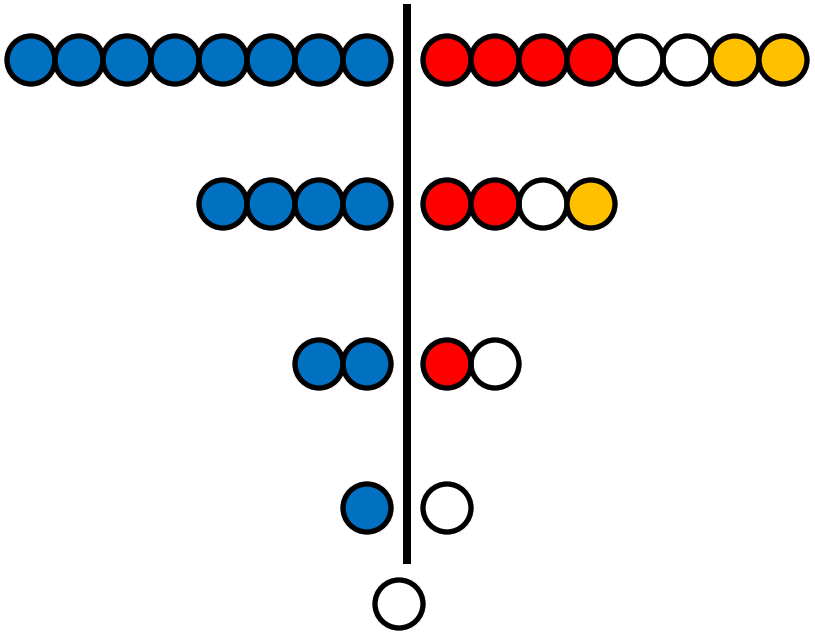
Colorectal Adenocarcinoma

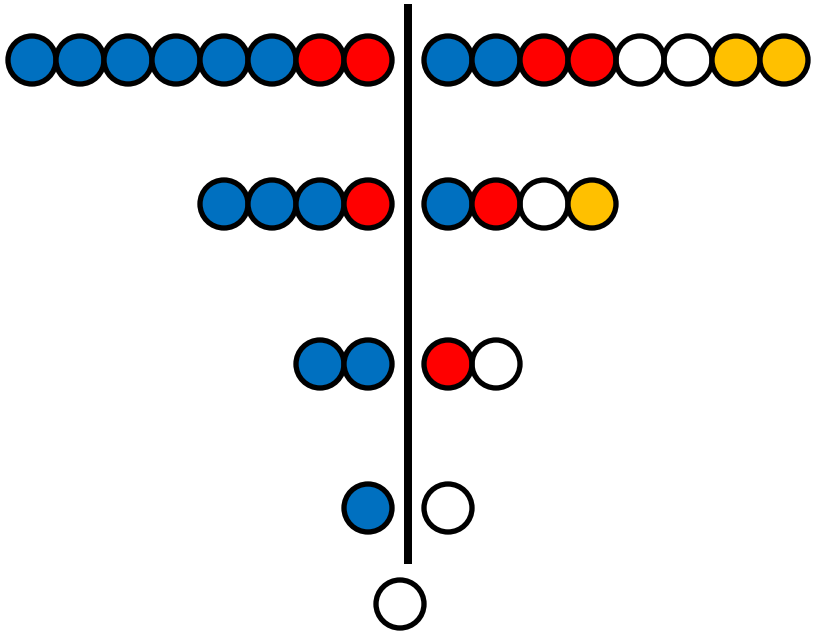




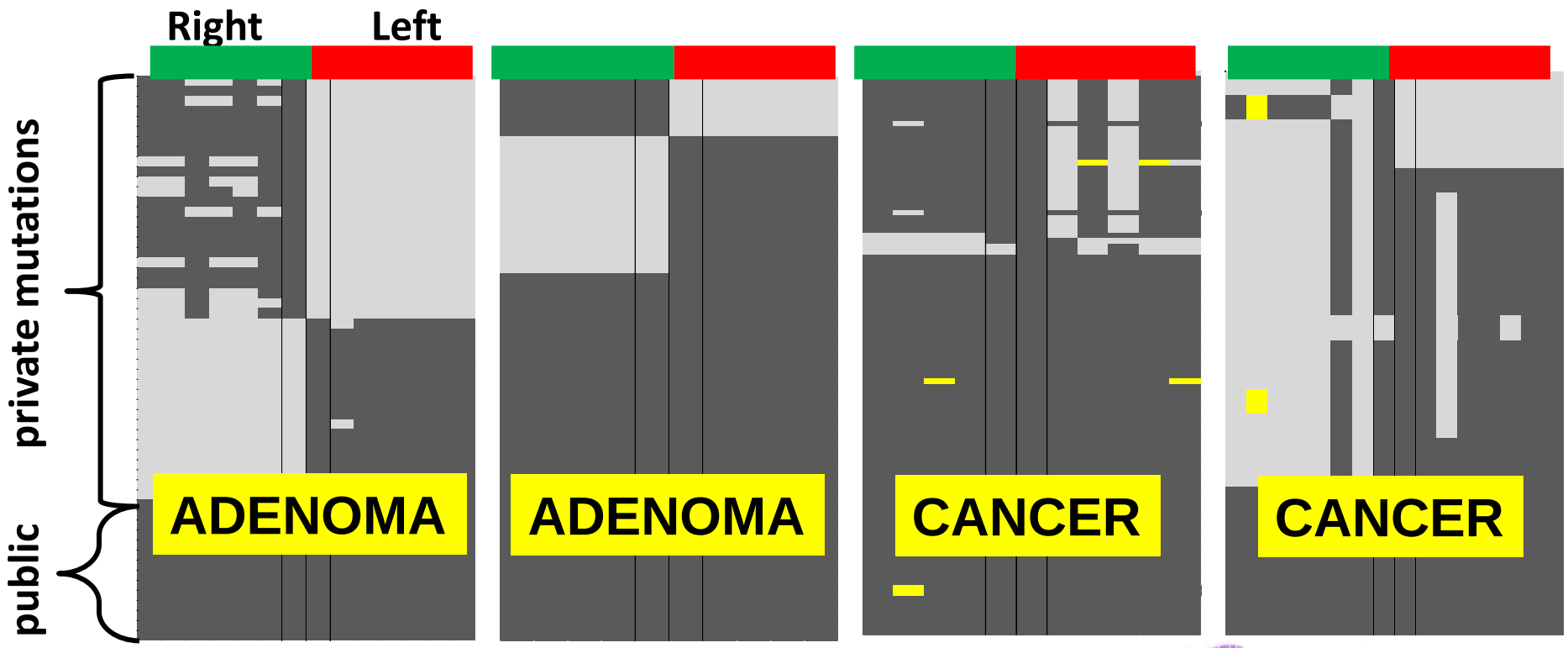
--Individual Cells Can Migrate
--Hard For Glands To Migrate

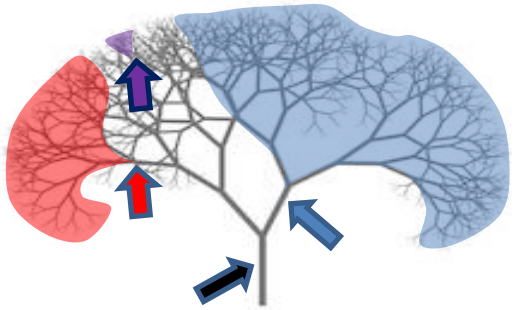
Detectability:
Human Tumors Are Large Versions Of Their Small Tumors

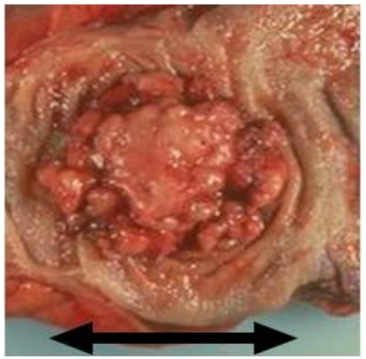


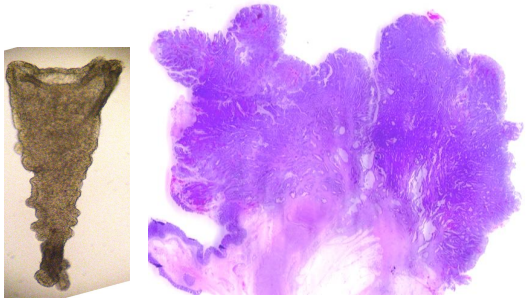


How Does A Private Mutation End Up On Both Sides Of A Tumor?







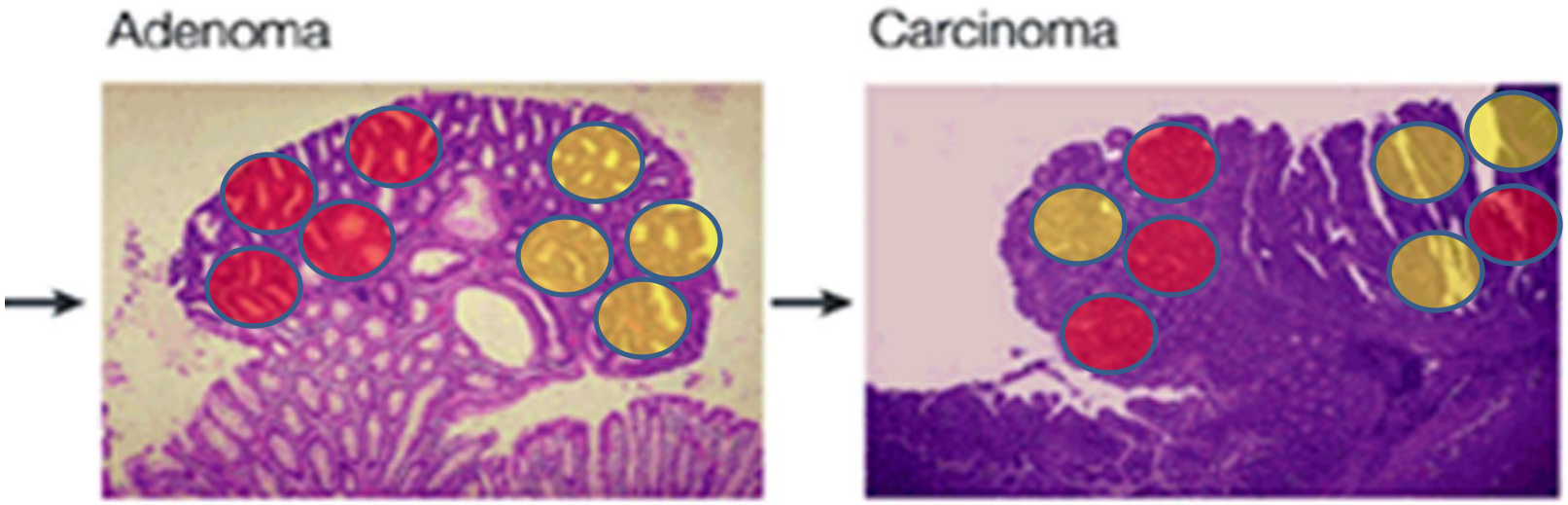


Cells Migrate But
Glands Don't Migrate Much

Colorectal Tumors

Benign Adenomas
(born to be good?)

Cancers:
Invasive and Metastatic
(born to be bad?)



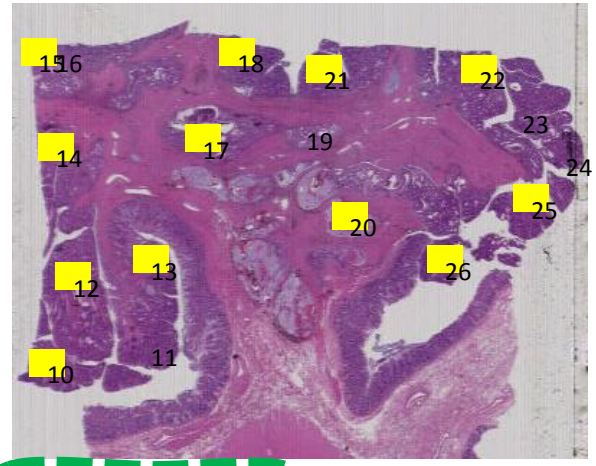
Mutation Patches

Mutation Polka Dots

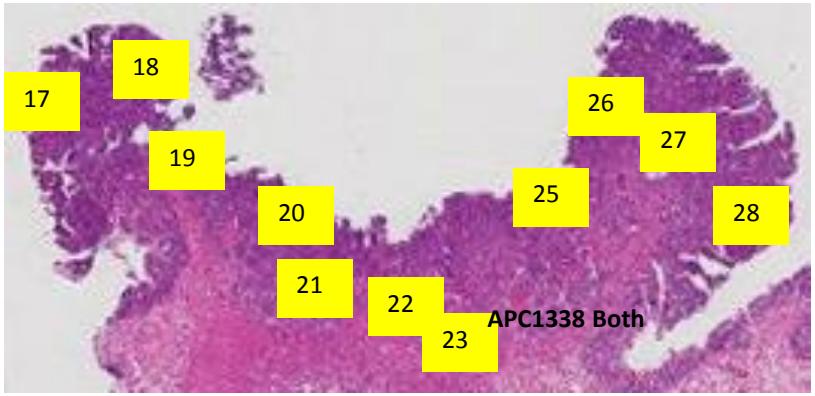
Adenoma

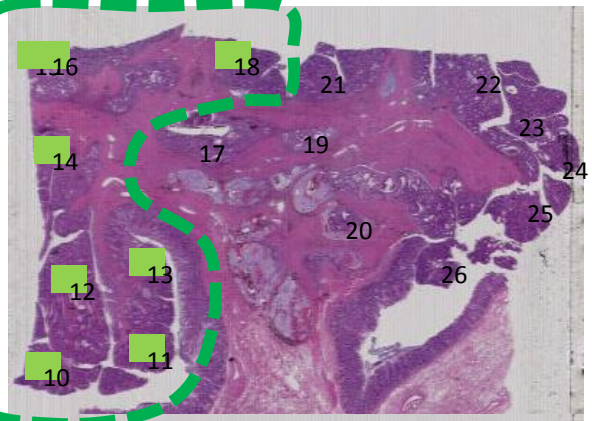
Microdissection Data

Cancer

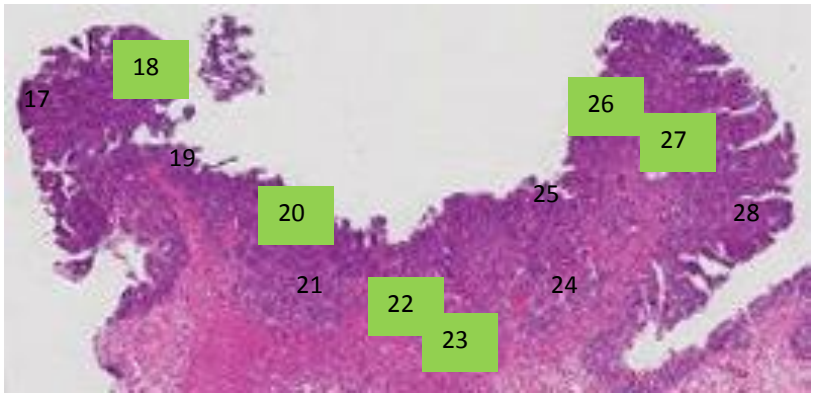


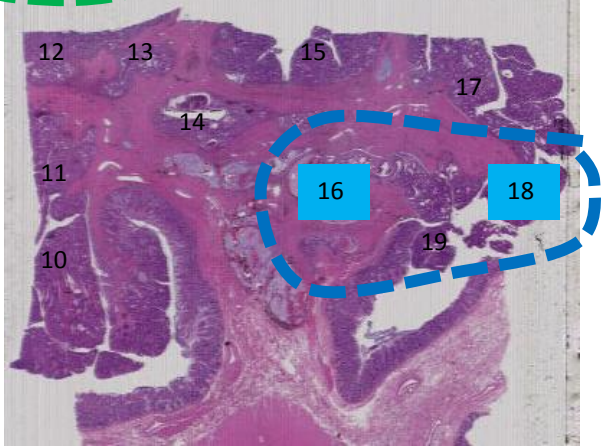
public mutation



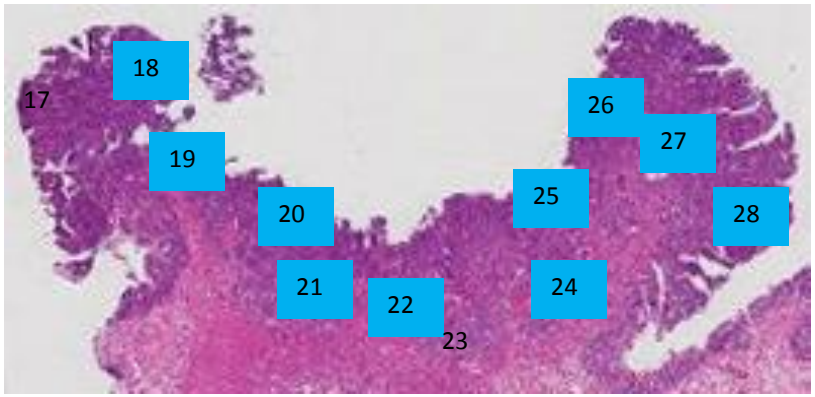


private mutation



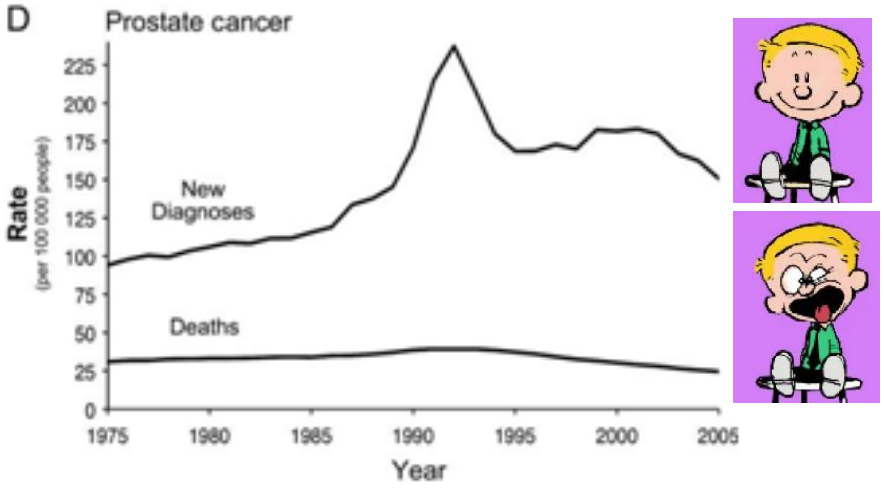


private mutation

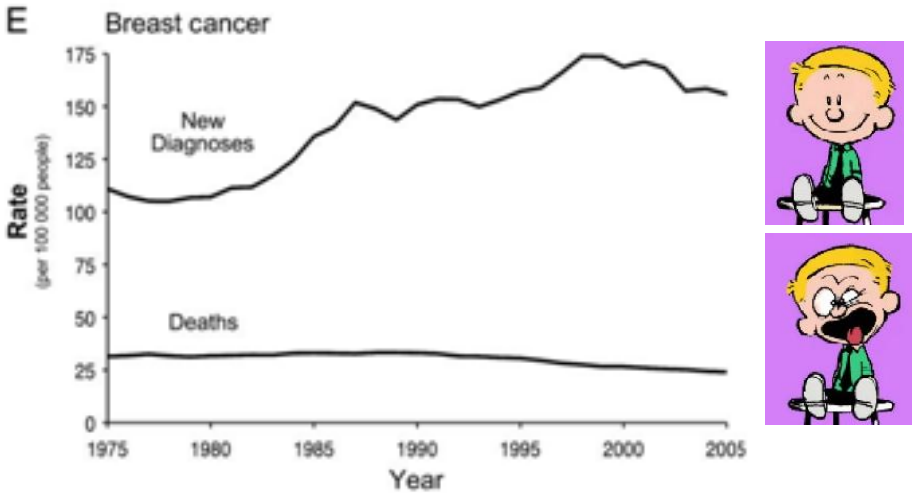


Difficult To Predict The Lethality Of Small Human Tumors

(lessons from screening)



Many Small Detected "Cancers"
Likely Will Not Kill Their Hosts



Potential To Distinguish
Early Lesions
"Born To Be Bad"
From those
"Born To Be Good"

Rate of new diagnoses and death in the Surveillance, Epidemiology, and End Results data from 1975 to 2005.

Published by Oxford University Press 2010.

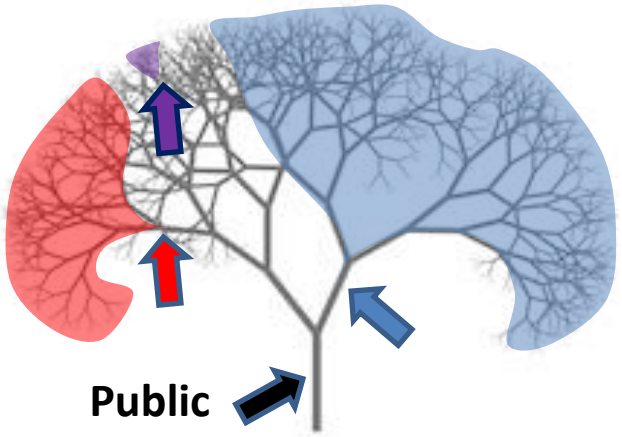
Welch H G , and Black W C JNCI J Natl Cancer Inst 2010;102:605-613

JNCI

MODEL ASSUMPTIONS/TESTABILITY/PROBLEMS/PROMISES

Assumptions

1) Start From Single Cell
2) Grows Into A Tumor (how fast?)
3) Presence or Absence of Early Mixing



Testability

1) Sample Single Glands
2) SNP microarrays
3) DNA sequencing
4) Specific Predictions

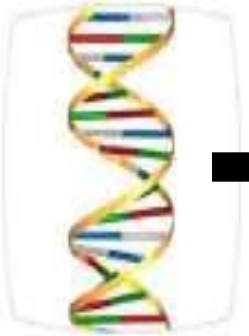
Problems

1) Implies A Burst Of Mutations During The First Few Divisions
   (Many Detectable Private Mutations)

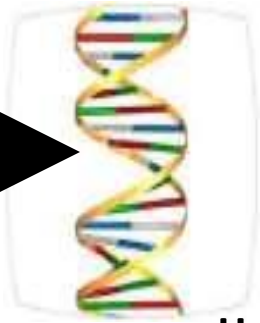
Promises

1) Implies The First Few Tumor Cells Divisions Are Unlike Any Other (Singularity)

Genomes Are “Historical” Documents (almost perfect copies of copies)



zygote
(start)



current cell
(end)

Acknowledgements

- Yasushi Yatabe
- Kyoung-Mee Kim
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- Peter Calabrese
- Kim Siegmund
- Paul Marjoram
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- Trevor Granham
- **Christina Curtis**
- **Andrea Sottoriva**