Reconstructing Human Cancer Progression From Private and Public Mutations

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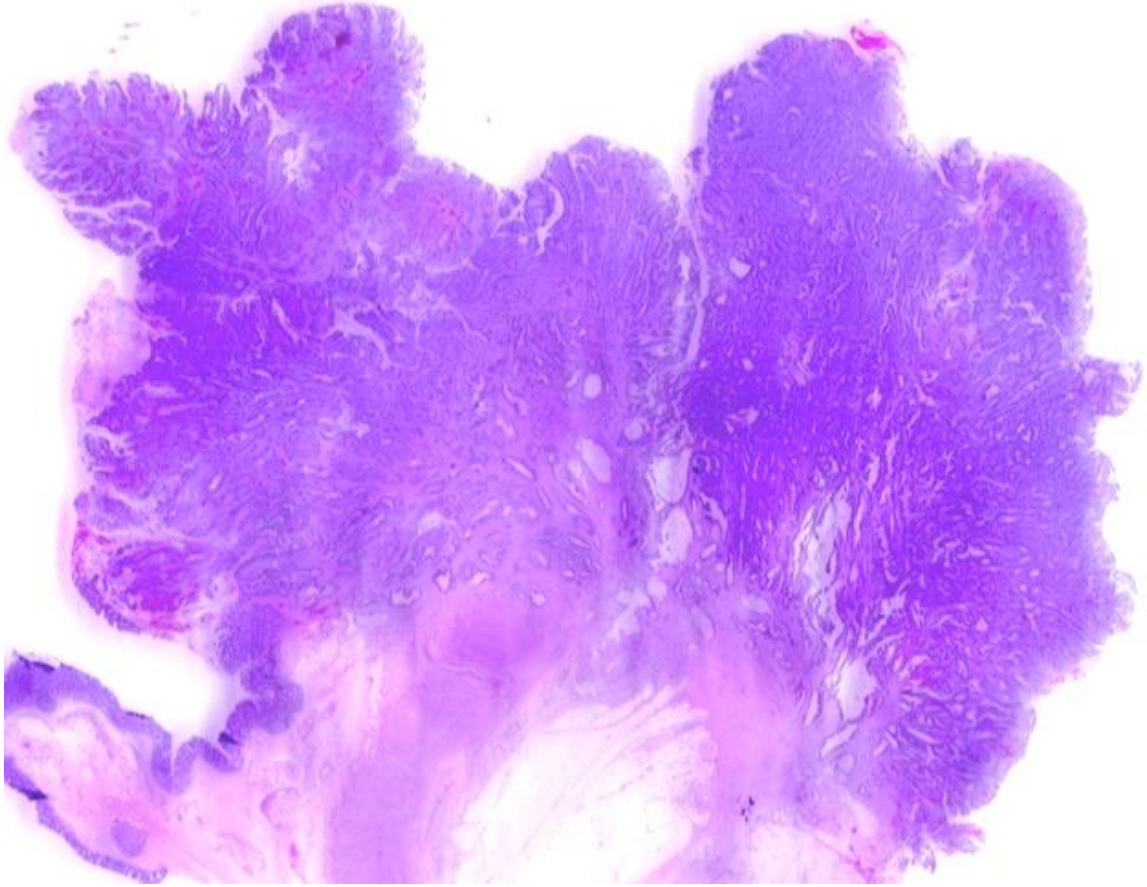
Model System: Human Colorectal Cancer

Specific Goal:

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

Clinical Question:

Are Tumors “Born To Be Bad”?



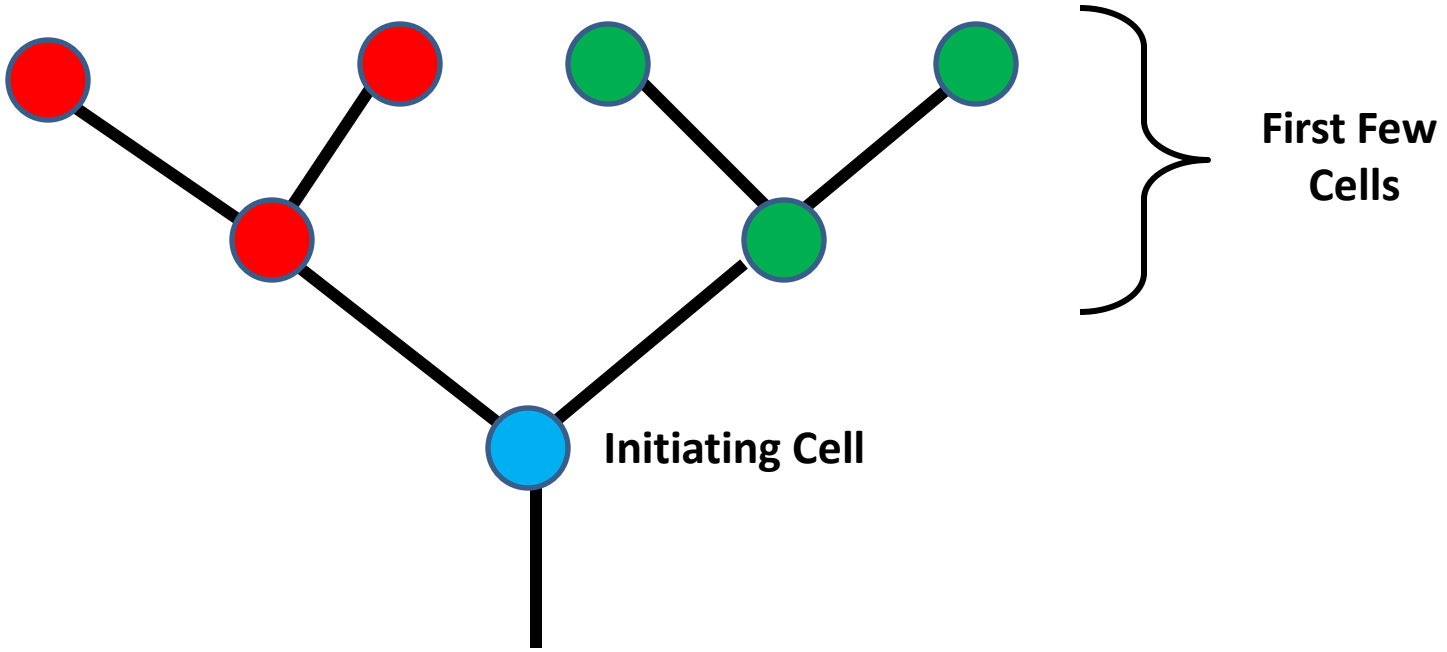
Are Human Tumors “Born To Be Bad”?

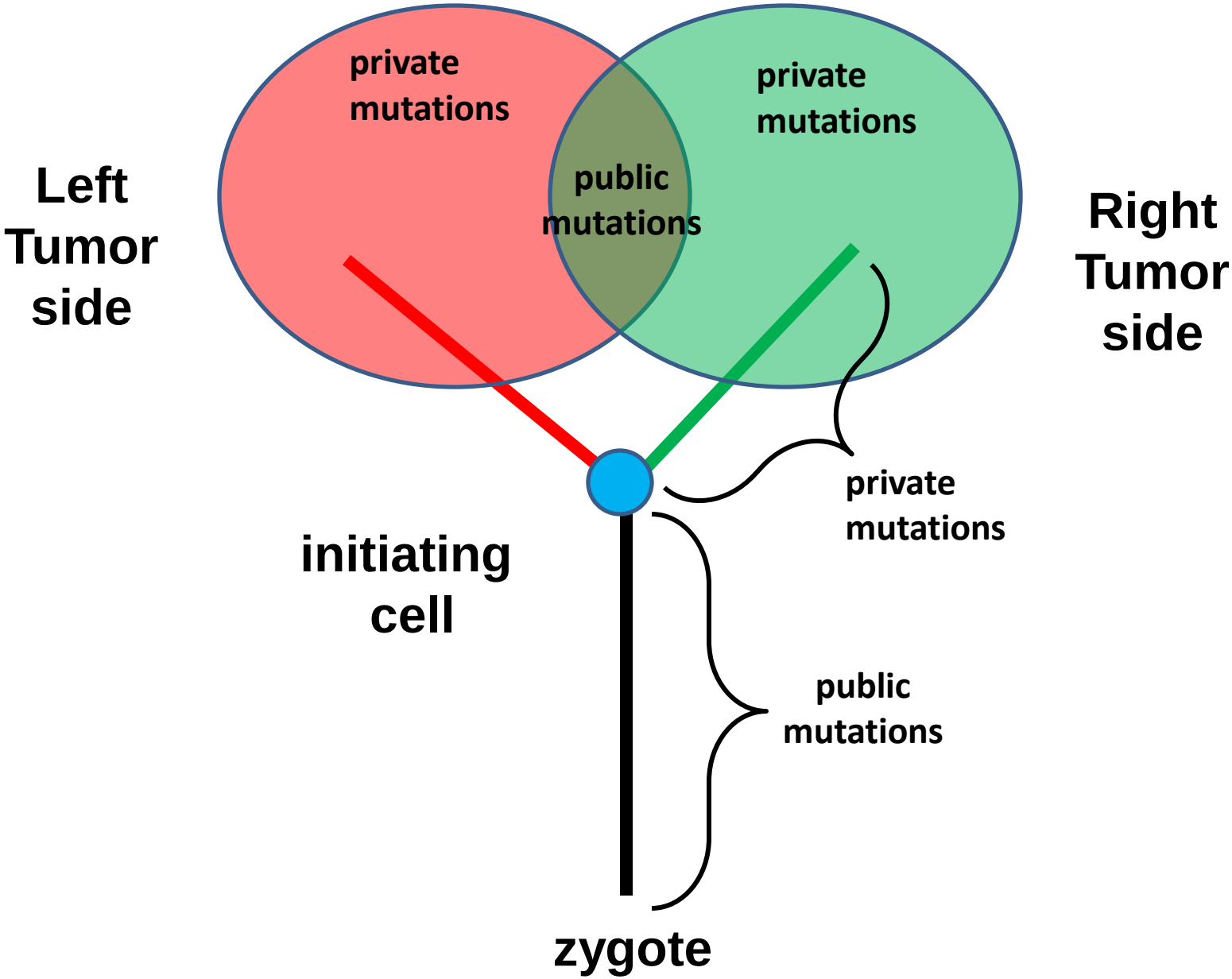
(Bernards,R.; Weinberg,R.A. A progression puzzle. Nature 2002, 418, 823)

Idea that the full malignant potential of a tumor is present at the time of initiation

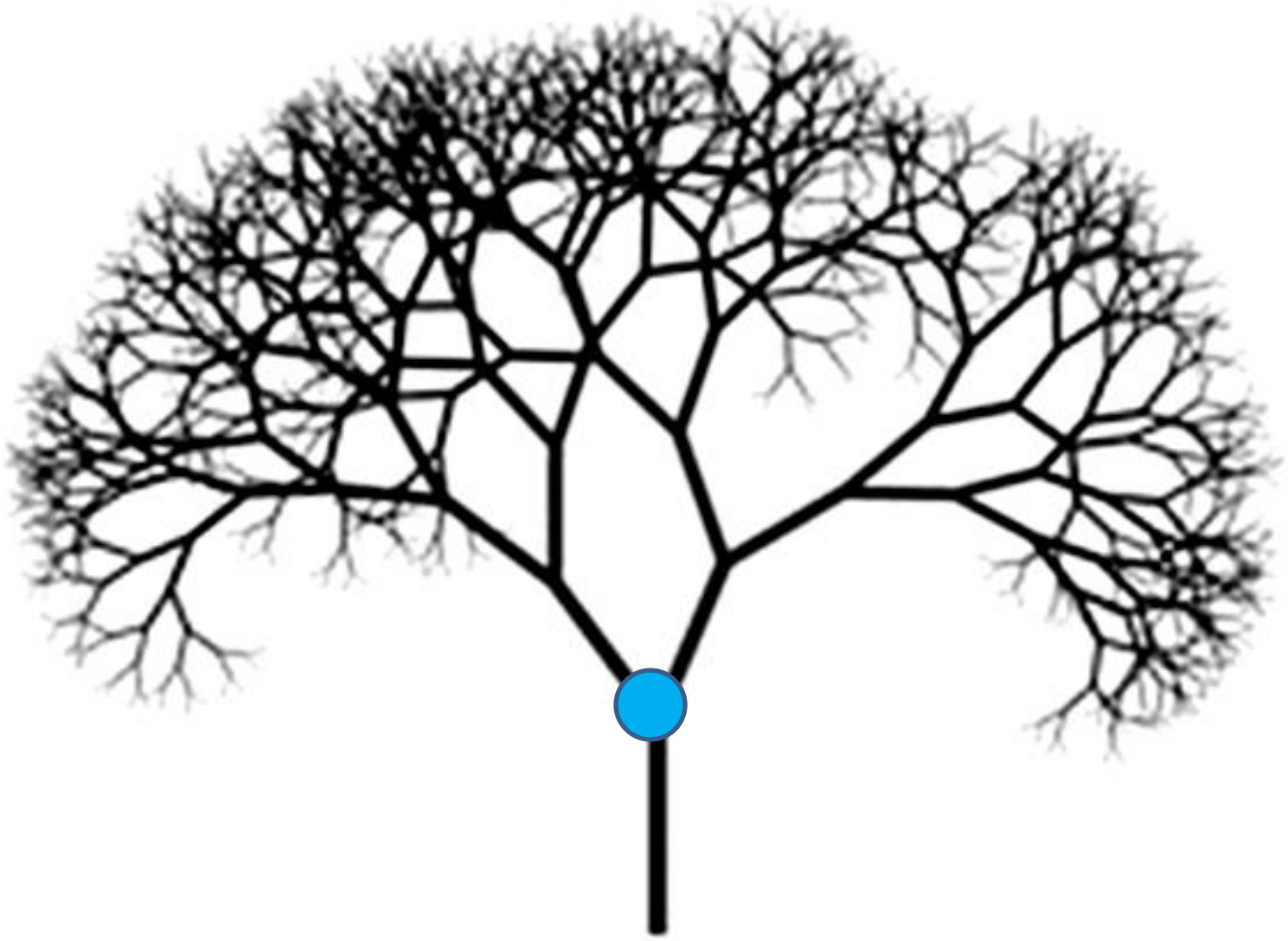
Experimental Strategy: (coalescence theory)

1) Define and Measure The First Few Tumor Cells (“Born”)
2) Define and Measure The Behaviors of The First Few Tumor Cells (“Bad”)




Left Tumor side
private mutations
public mutations
private mutations
Right Tumor side
private mutations
initiating cell
public mutations
zygote

Complex Ancestral Somatic Cell Tumor Tree



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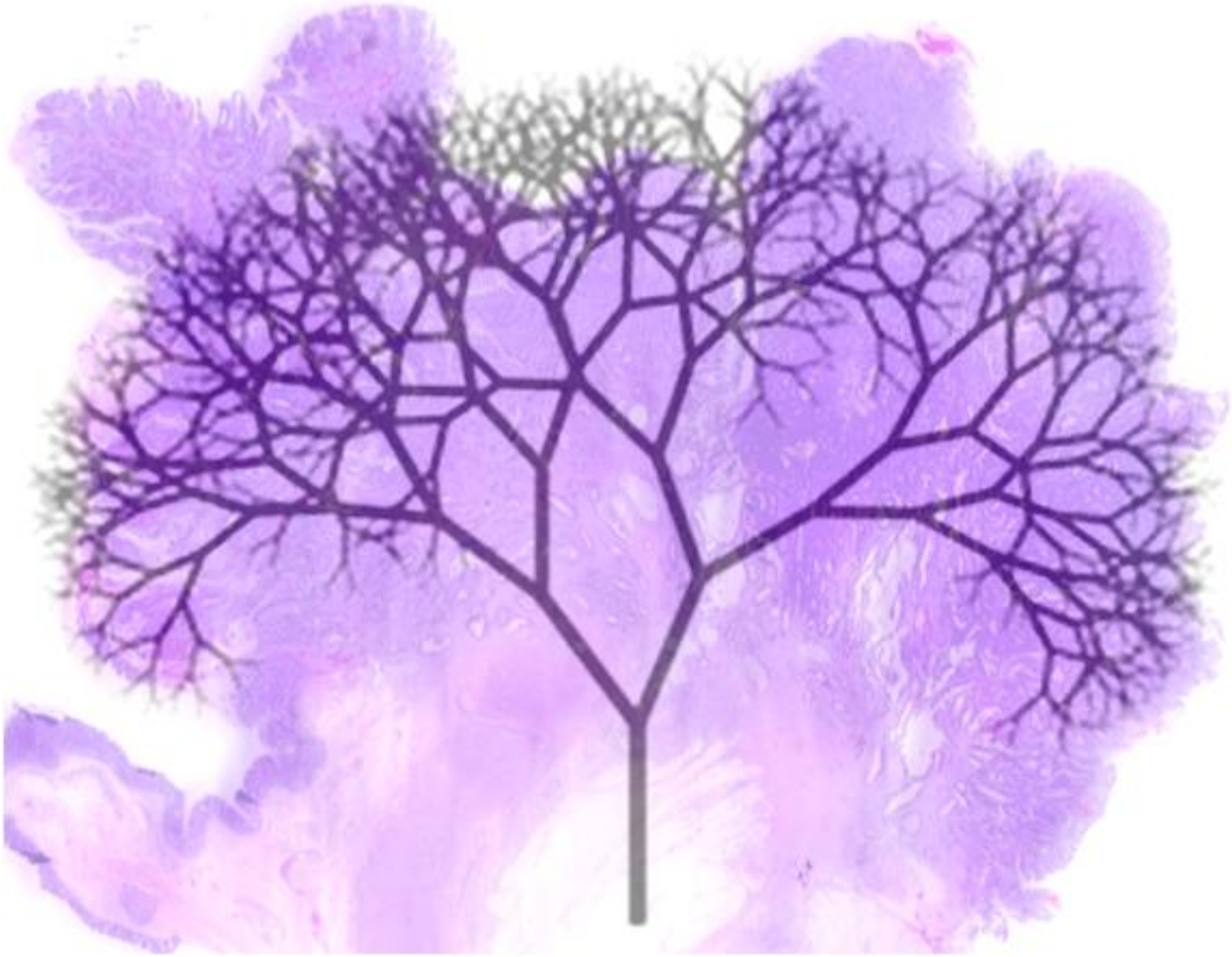
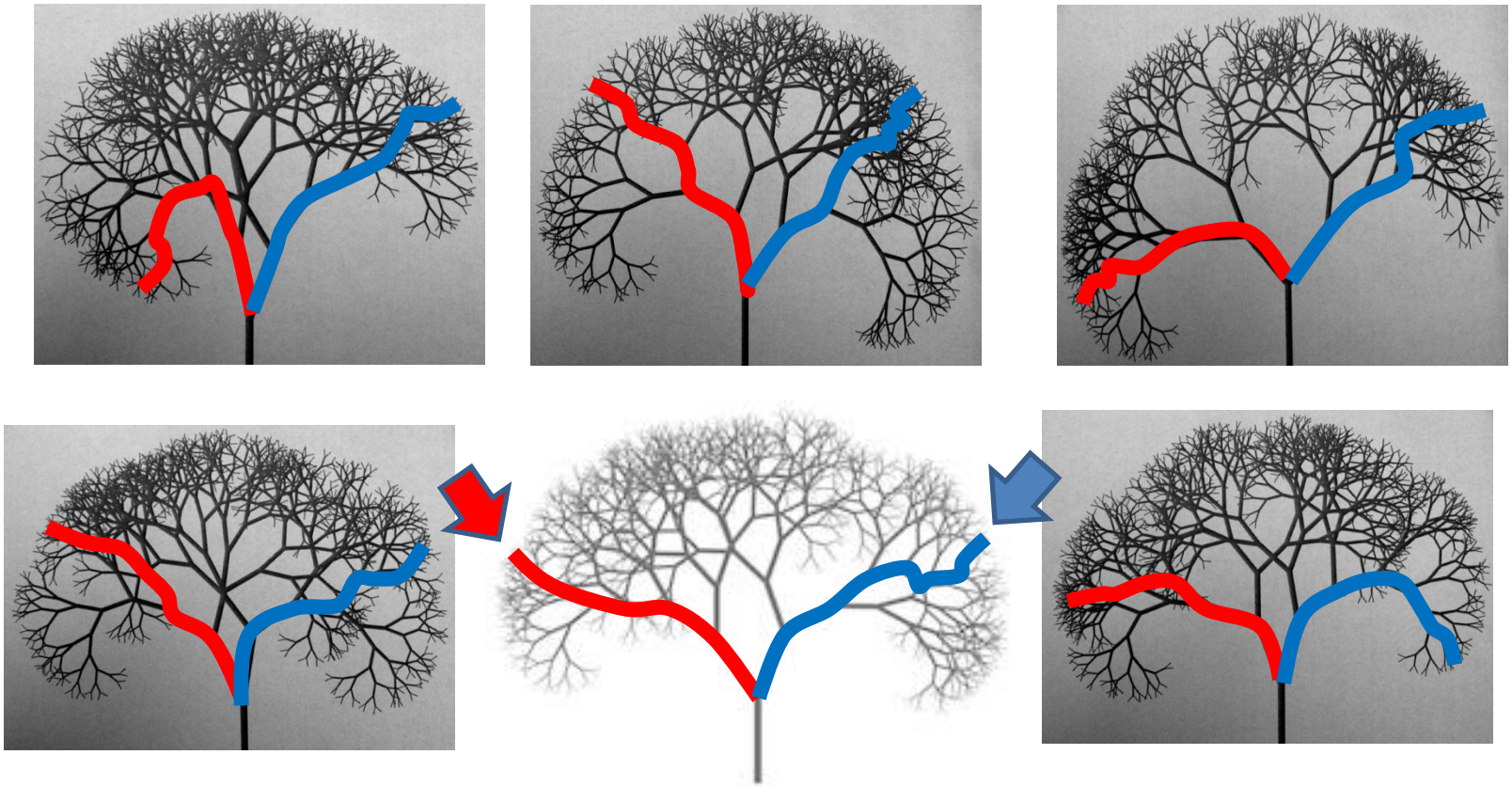


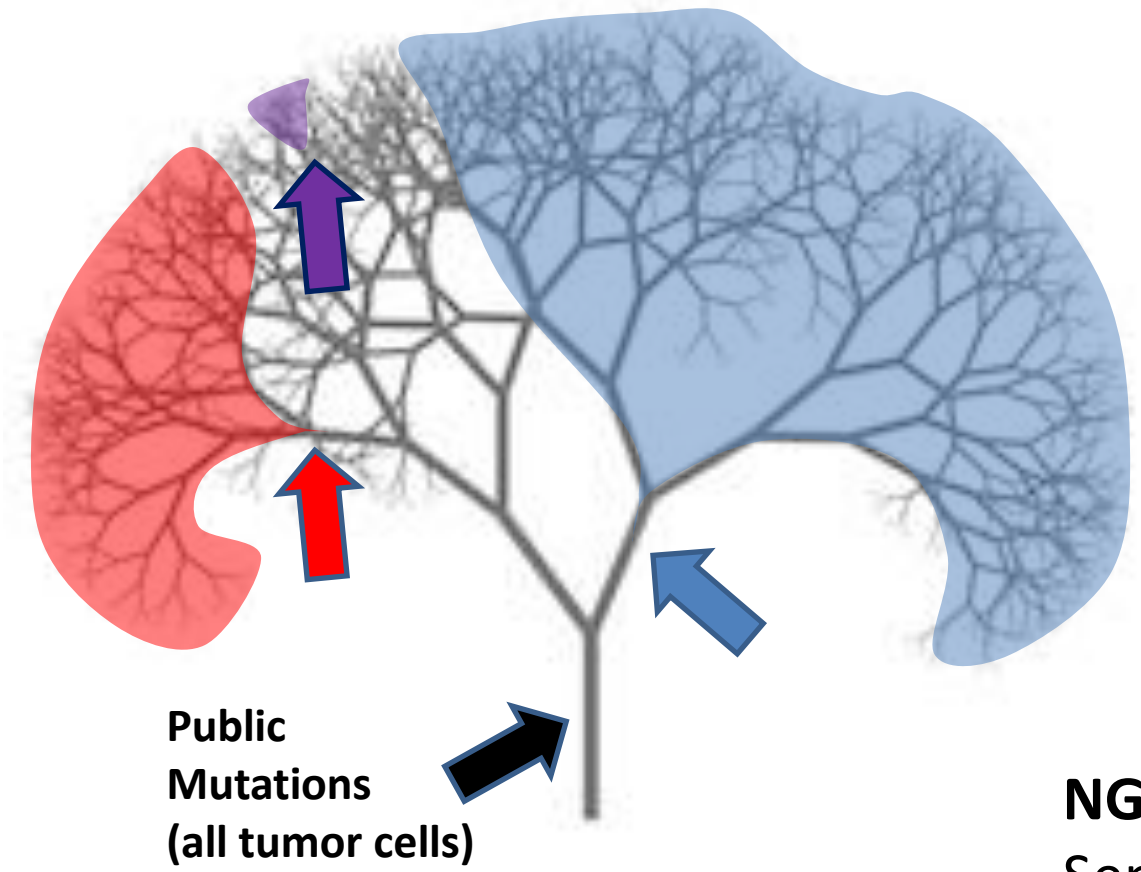
Many Possible Binary Trees:
BUT Early Tree Structure Relatively Easy To "Measure"



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

Early Private Mutations:

1) Easy To Sample
2) Easy To Detect

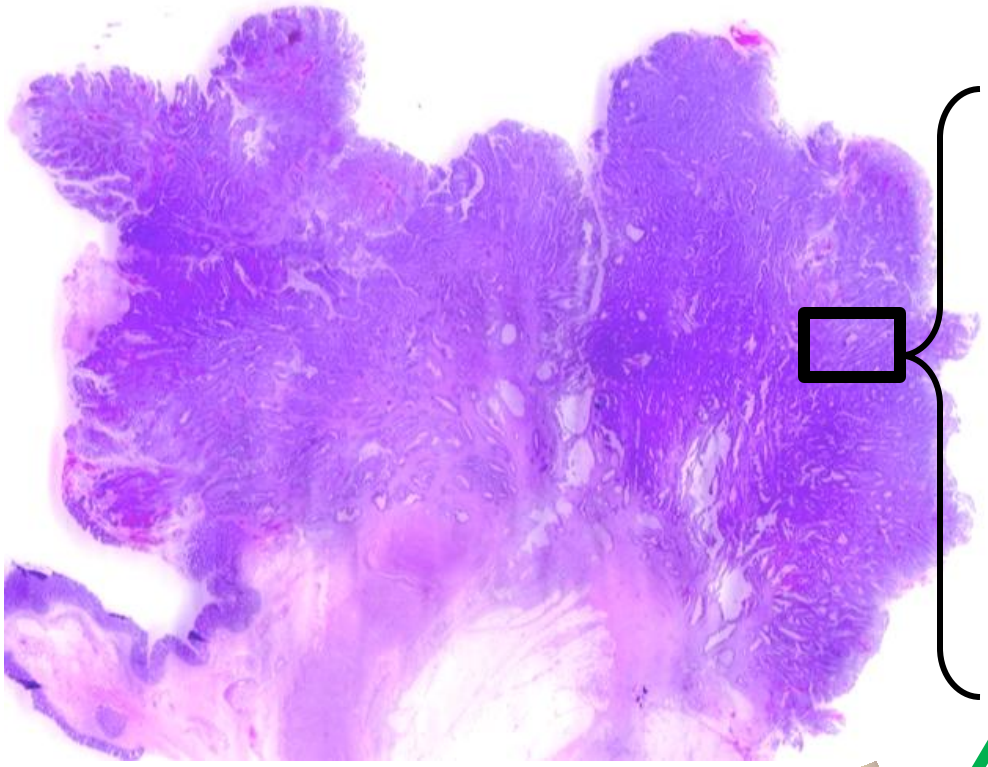


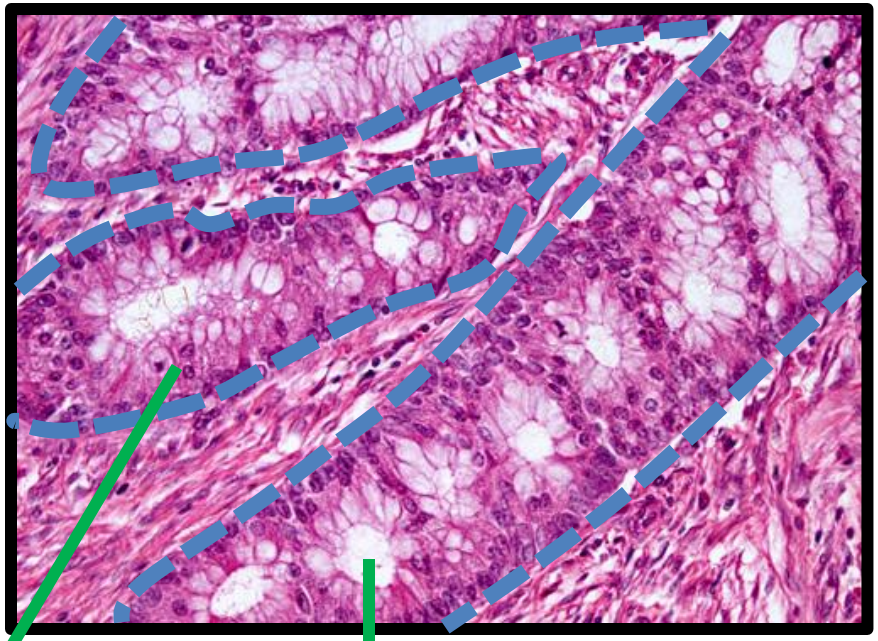
simple exponential expansion

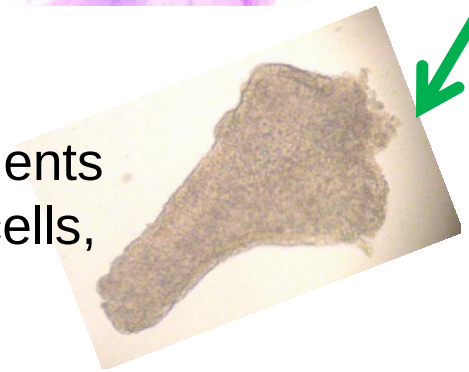
Public: 100% cells
Private:
Division 1: 50%
Division 2: 25%
Division 3: 12.5%
Division 4: 6.25%
Division 5: 3%

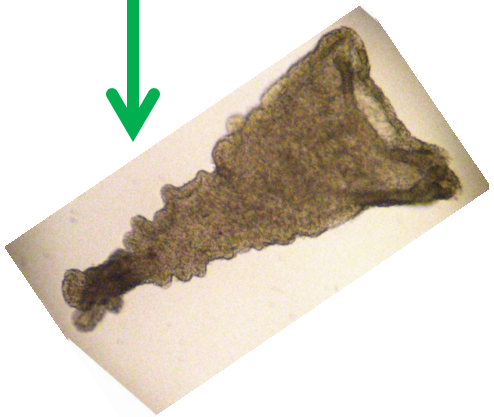
NGS Platforms:
Sensitivity About 10%
Mutation Frequency

Colorectal Cancers Have Structure (Adenocarcinomas With Glands)



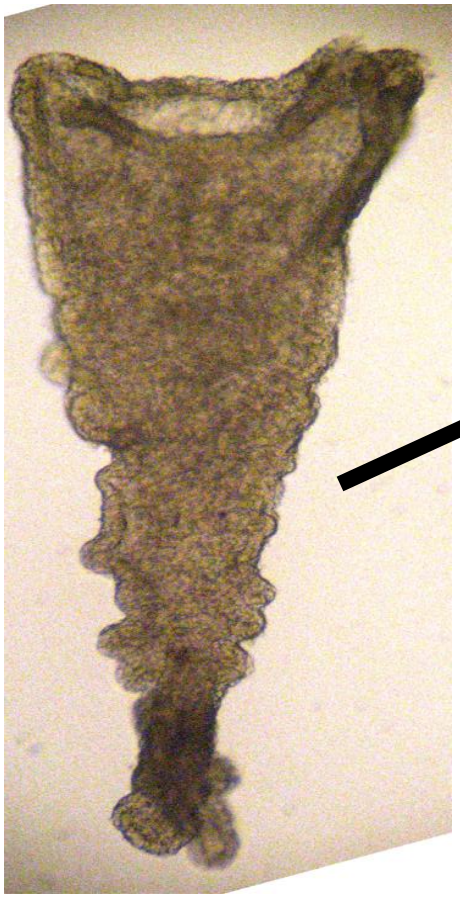






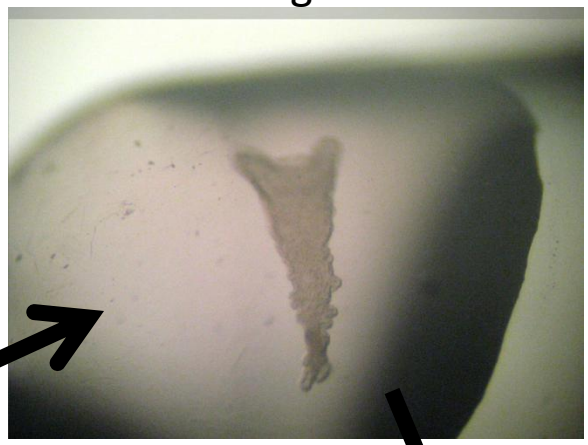
Tumor Gland Fragments
(~10,000 adjacent cells,
>95% pure)

Single Tumor Gland/Fragment 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)

Sampling Different Physical Scales

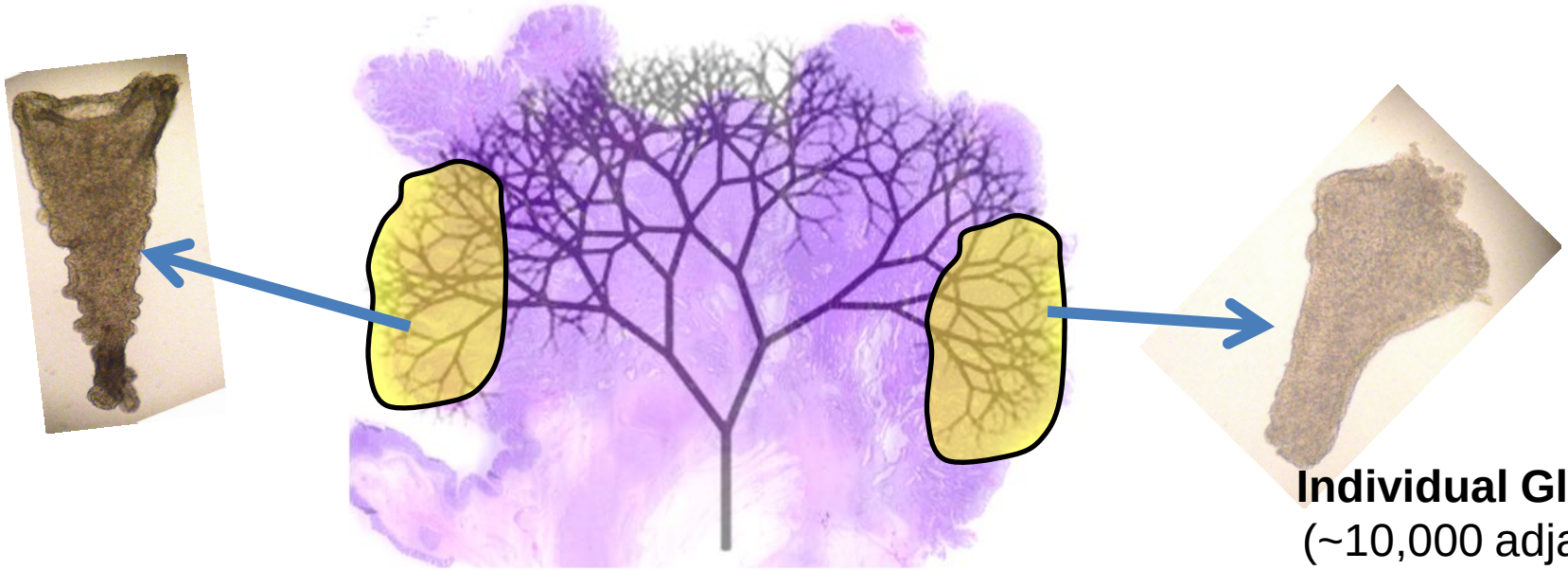
Whole Tumor (NGS, CNV (SNP-chips))

Tumor Half (NGS, CNV)

Individual Glands

(targeted sequencing, CNV, DNA methylation)

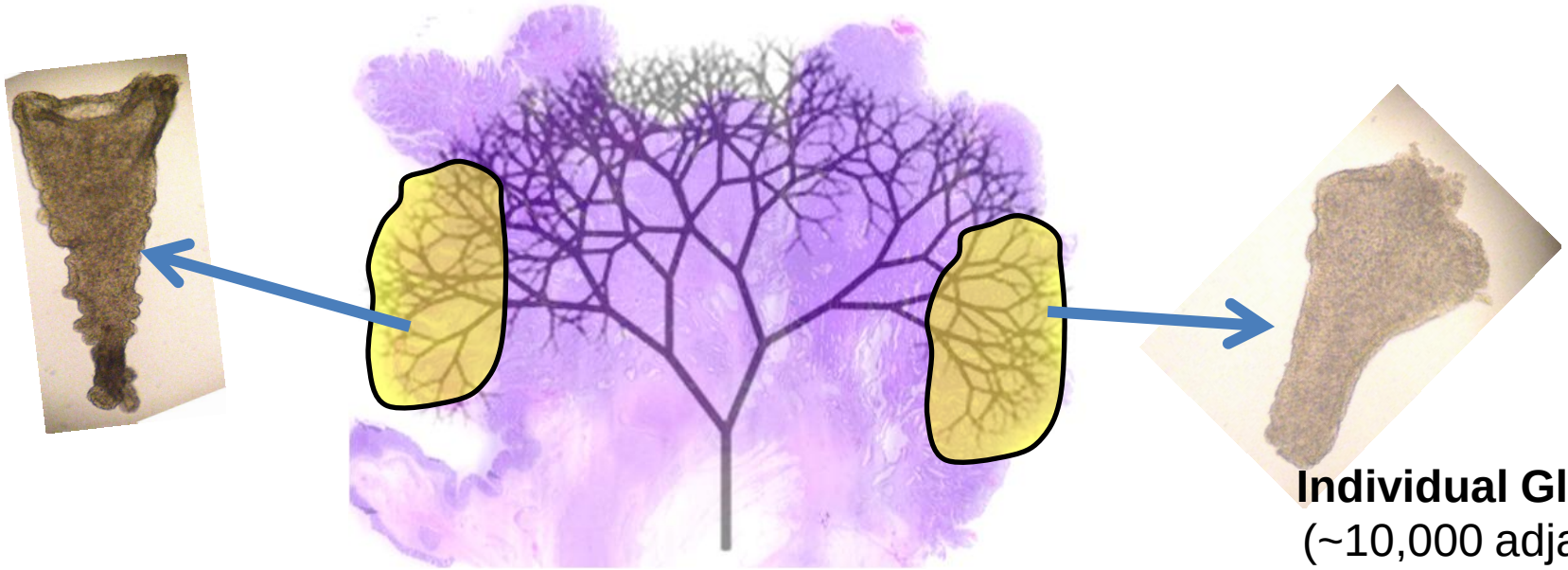
Individual Cells (FISH)



Individual Glands
(~10,000 adjacent cells)

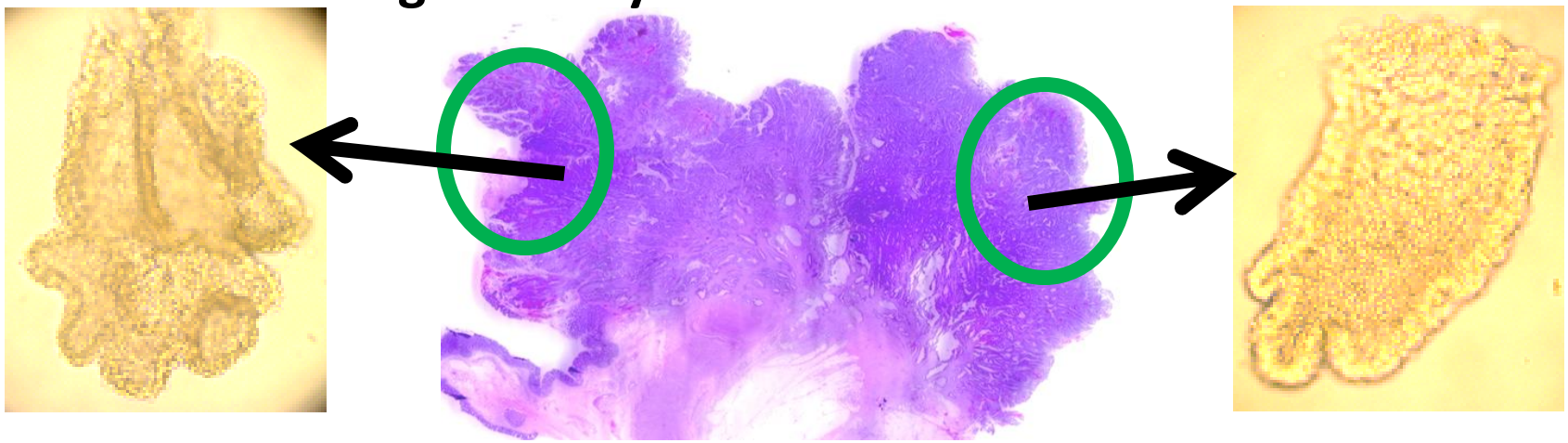
Relative Error and Mitotic Rates ("molecular clocks")

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	Stepwise Changes



Individual Glands
(~10,000 adjacent cells)

Experimental Strategy: Sample Multiple Tumor Glands DNA Passenger Methylation Patterns



six cancer glands

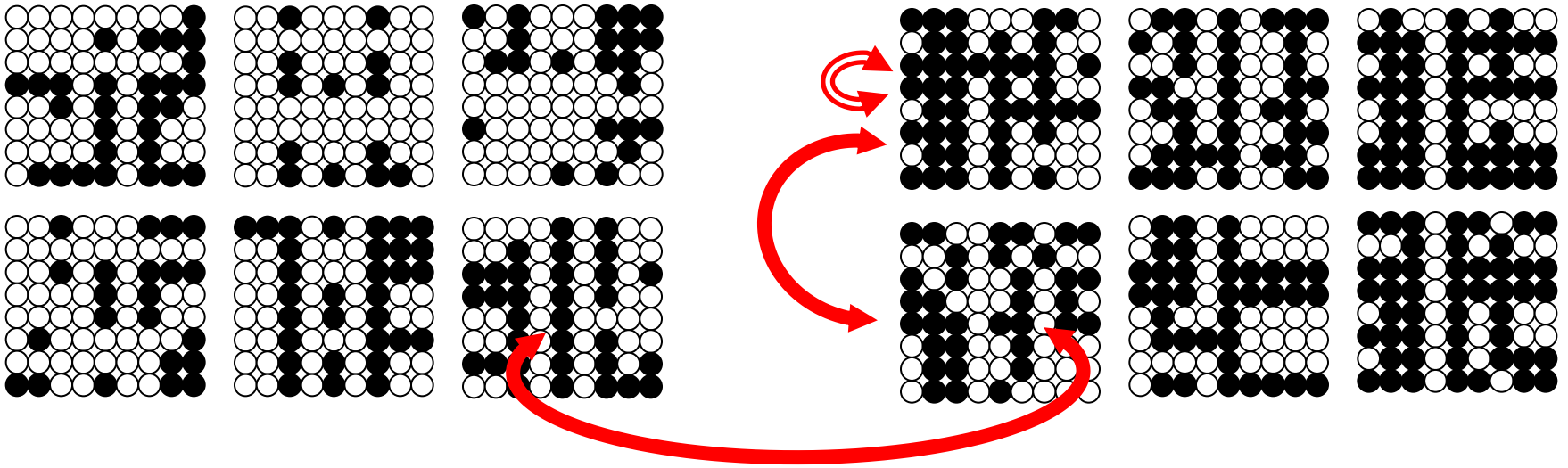
left side

5′ 3′ 5′ 3′ 5′ 3′

six cancer glands

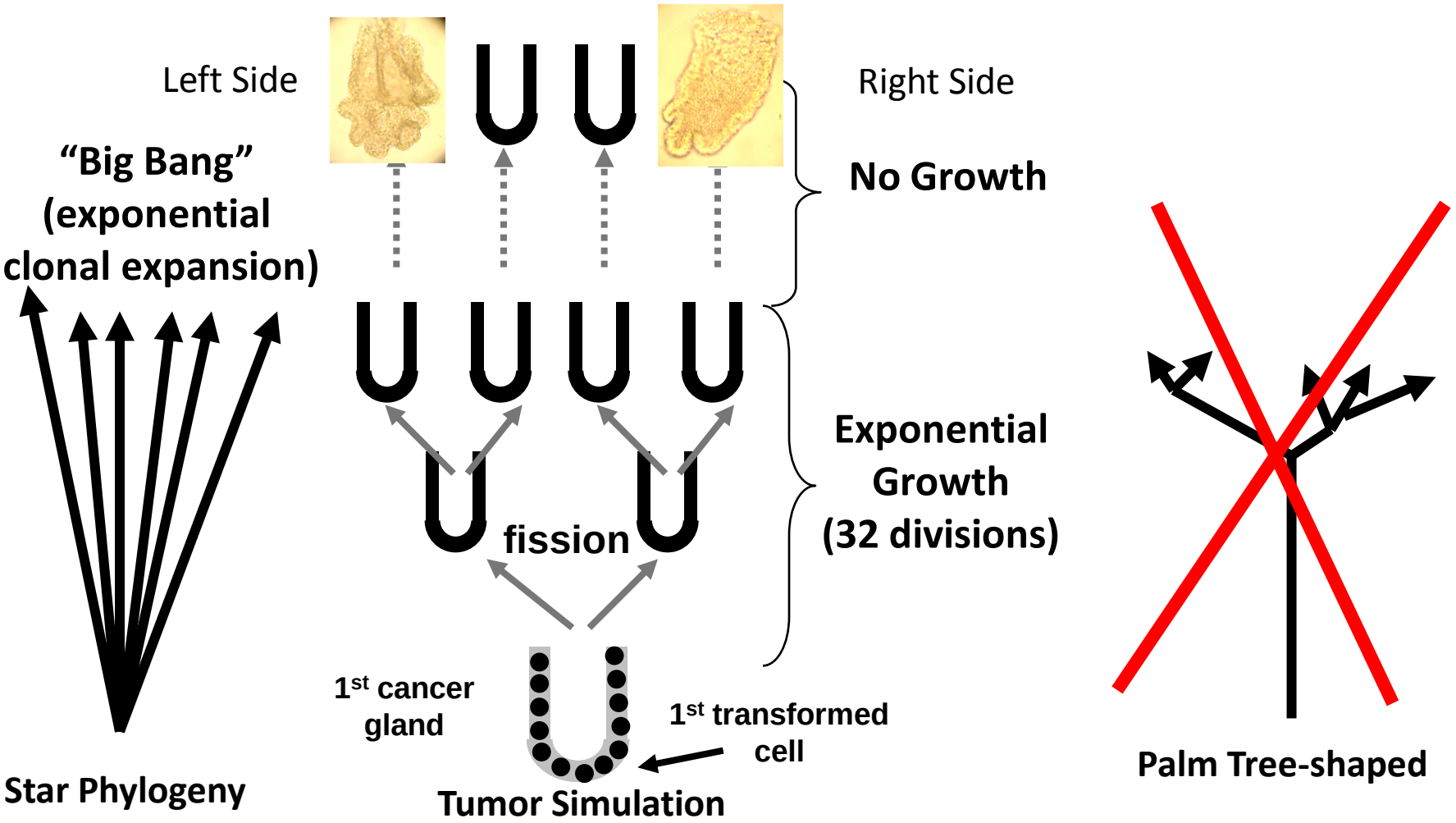
right side

5′ 3′ 5′ 3′ 5′ 3′

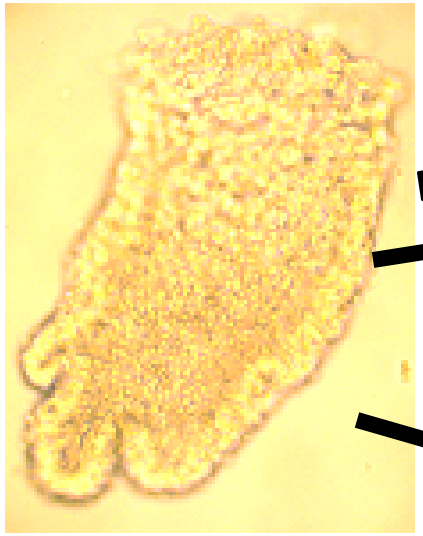


Passenger DNA Tumor Gland Methylation:
More Consistent With A Star Phylogeny (single clonal expansion)

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)

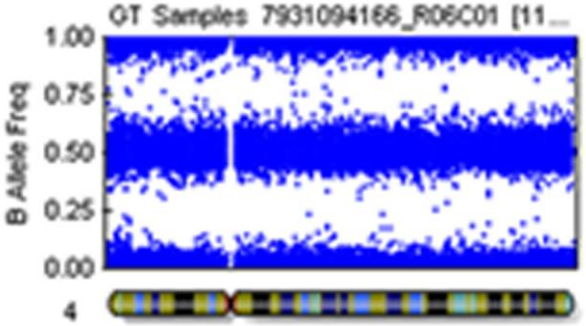


Chromosome CNAs
(Chromosomal Instability (CIN))



Gland

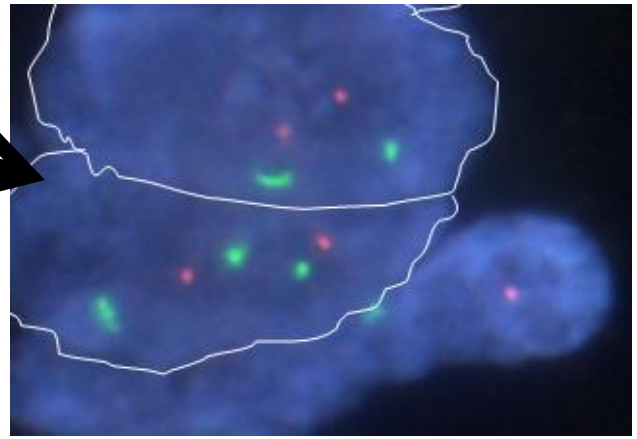
Examine Single Gland



SNP Microarray:

<u>Average</u> of gland = "Diploid"

Examine Single Cells



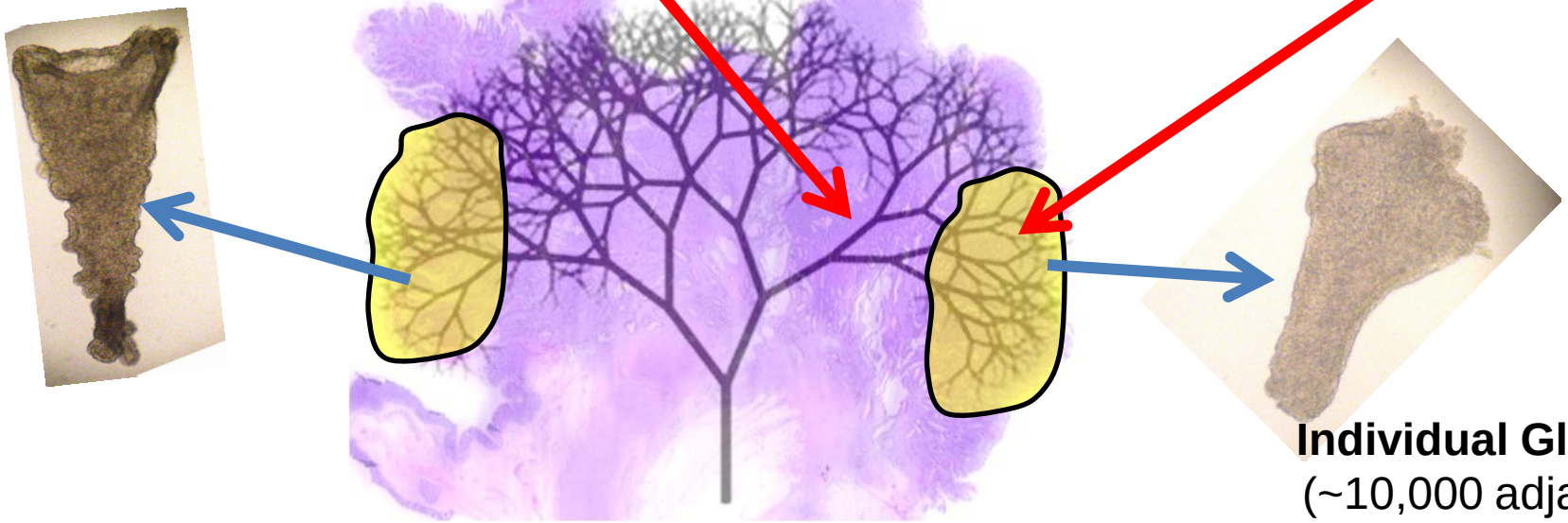
FISH: CIN PRESENT
(different ploidy)

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

Relative Error and Mitotic Rates ("molecular clocks")

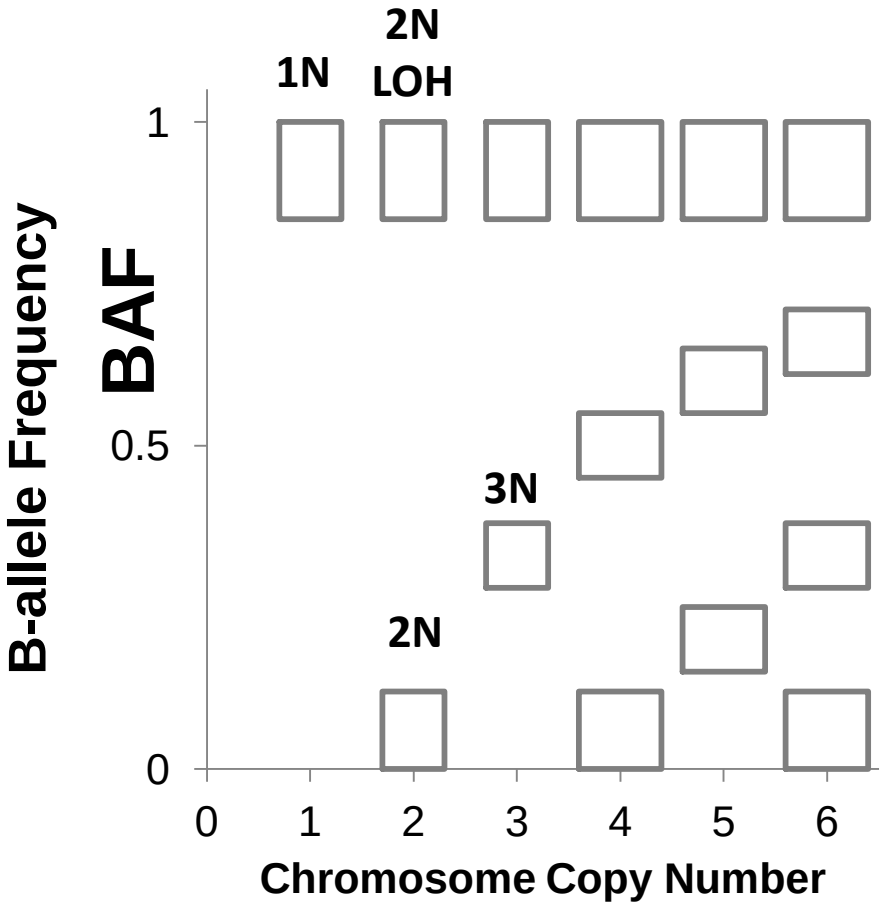
DNA base fidelity	~10^{-9} per base per division	
DNA methylation	~10^{-5} per base per division	Stepwise Changes
Chromosome CNA	~10^{-2} to 10^{-4} per division	

Early CNAs Are More Detectable Than Later CNAs With SNP microarrays

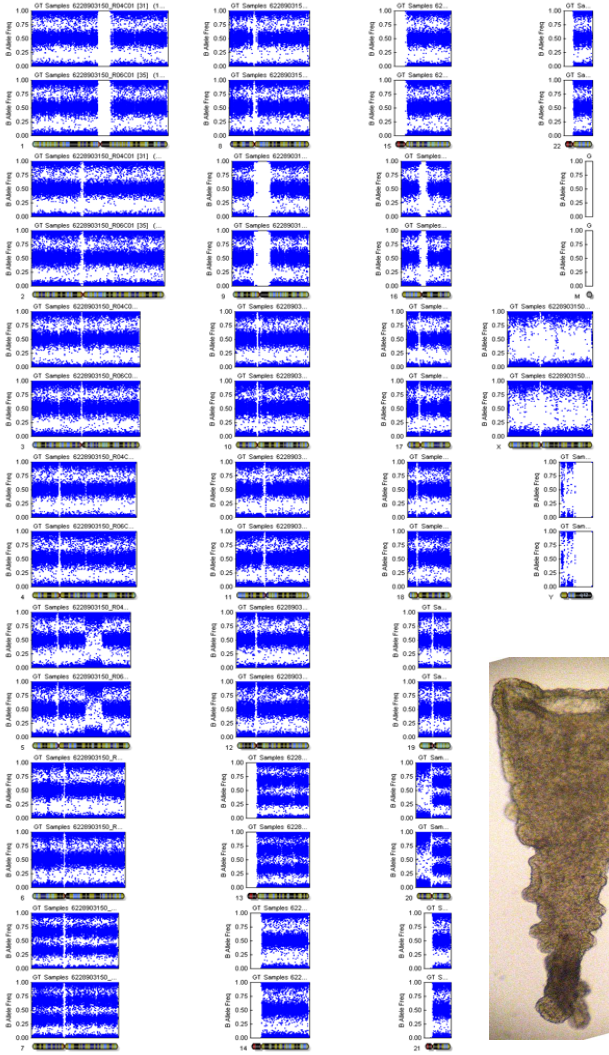


Individual Glands (~10,000 adjacent cells)

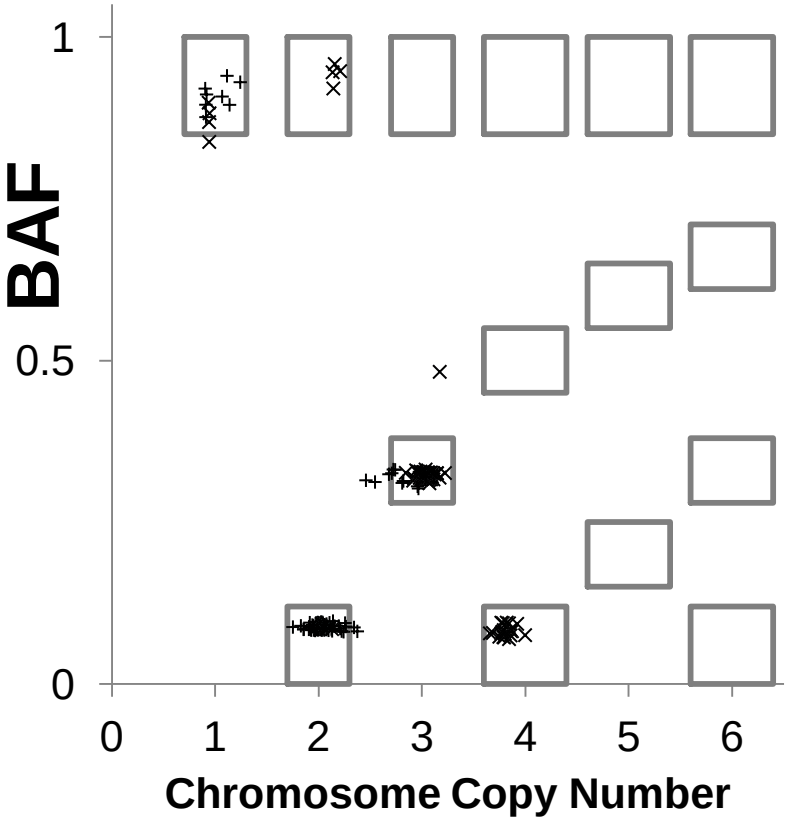
Visualizing Gland Chromosome Ploidy
("quantum normal values")



SNP microarrays

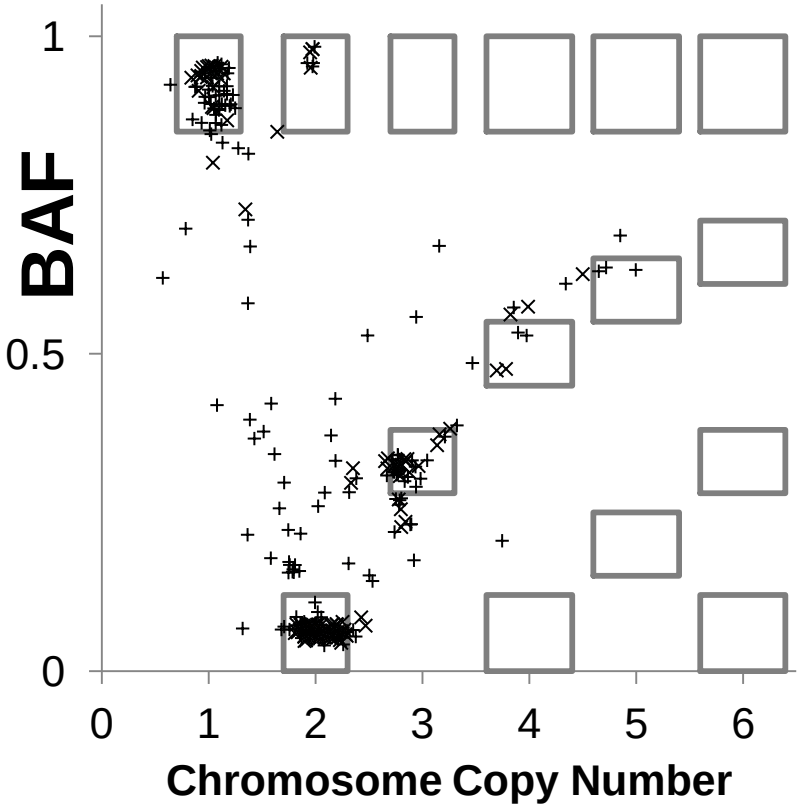


Despite "CIN" Most Gland Chromosome Fragments Are "Fixed" (near "quantum" or integer values)



Adenoma

(8 glands)

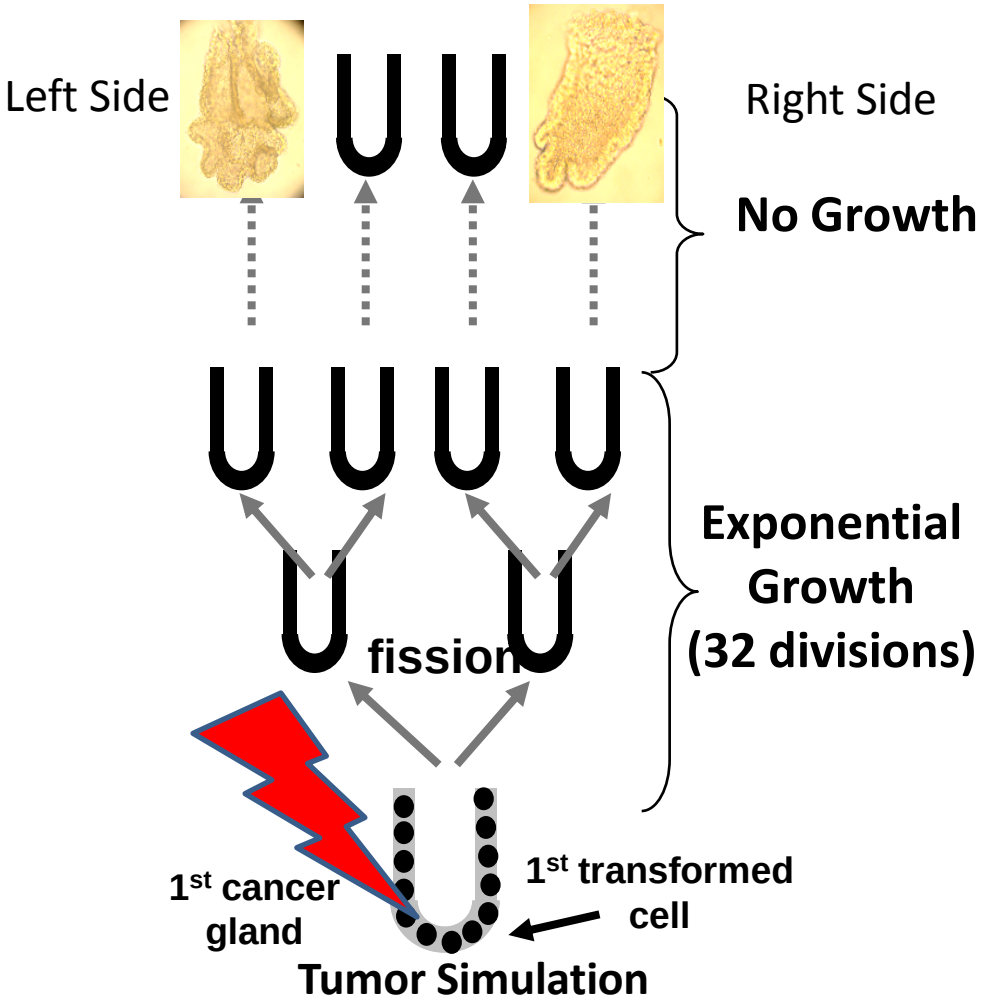


Cancer

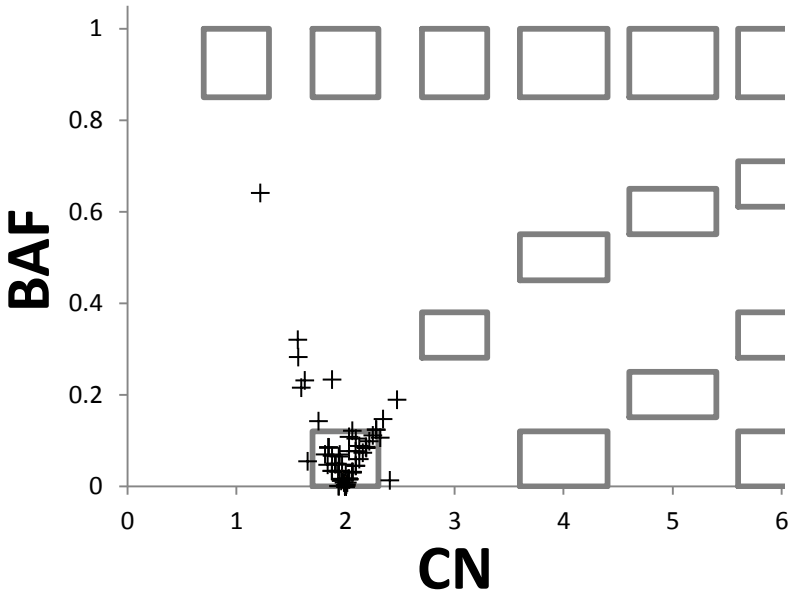
(8 glands)

Tumor Simulations of CIN

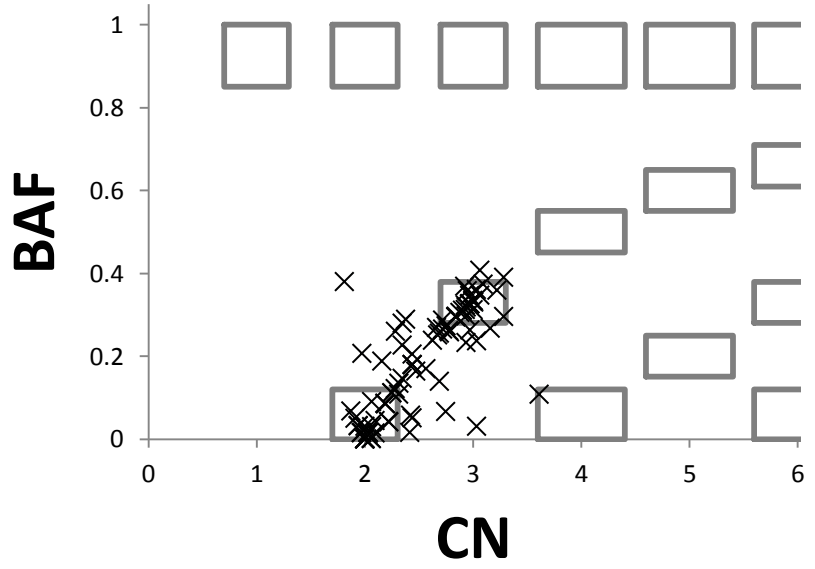
Start: 2 chromosomes,
200 divisions



Random CNA

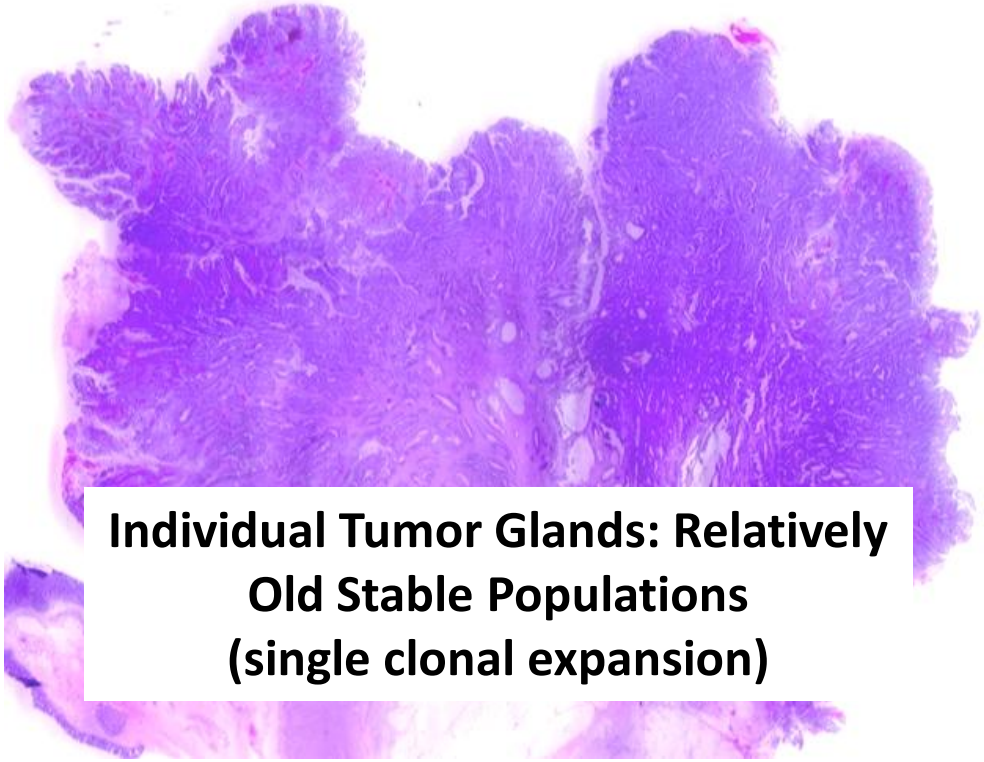


Force CNA (+1) At First Division



Summary of Tumor Gland Alterations

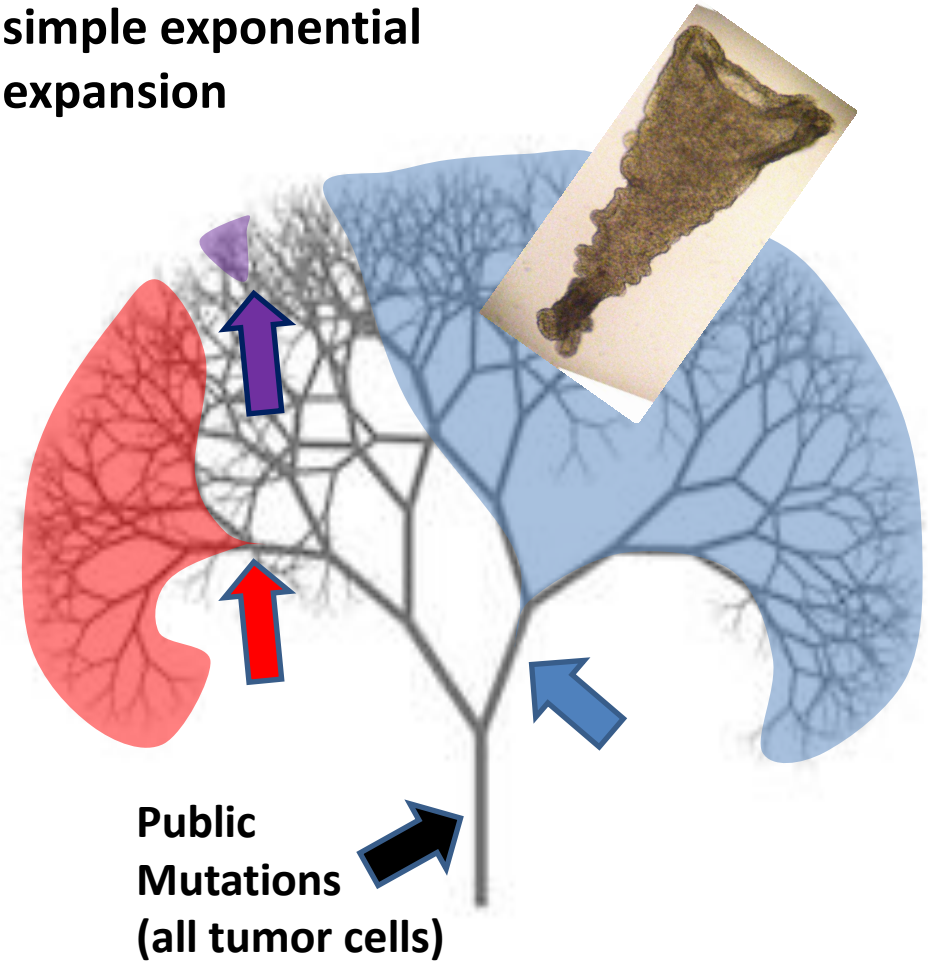
1) Passenger Methylation Patterns: Diverse
2) FISH Chromosome CNAs: Diverse
3) SNP Microarray: Many Average Gland CNAs Are “Quantum”



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%

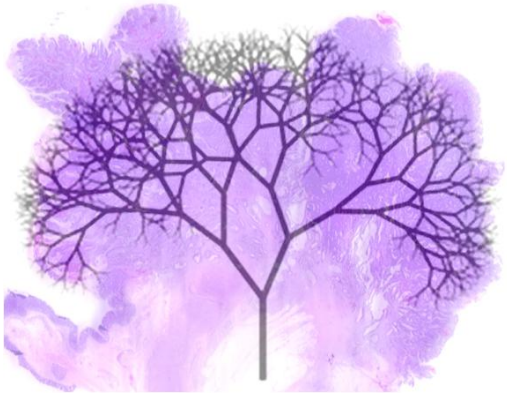
Single Gland

Public: 100% cells
Private:
Division 1: 100%
Division 2: 100%
Division 3: 100%
Division 4: 100%
Division 5: 100%??

Possible Gland Point Mutation Frequencies

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

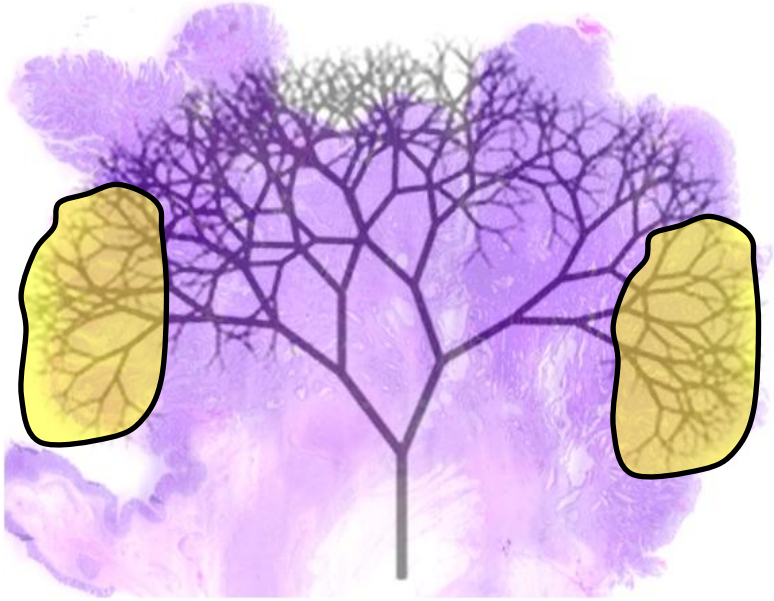
2) "Quantum" Values (1N, 2N, 3N…….)
----<u>Detectable</u> Mutations Are Public
and Early Private Mutations
----Individual Glands Are Old, Stable Populations
(fixation or lost)

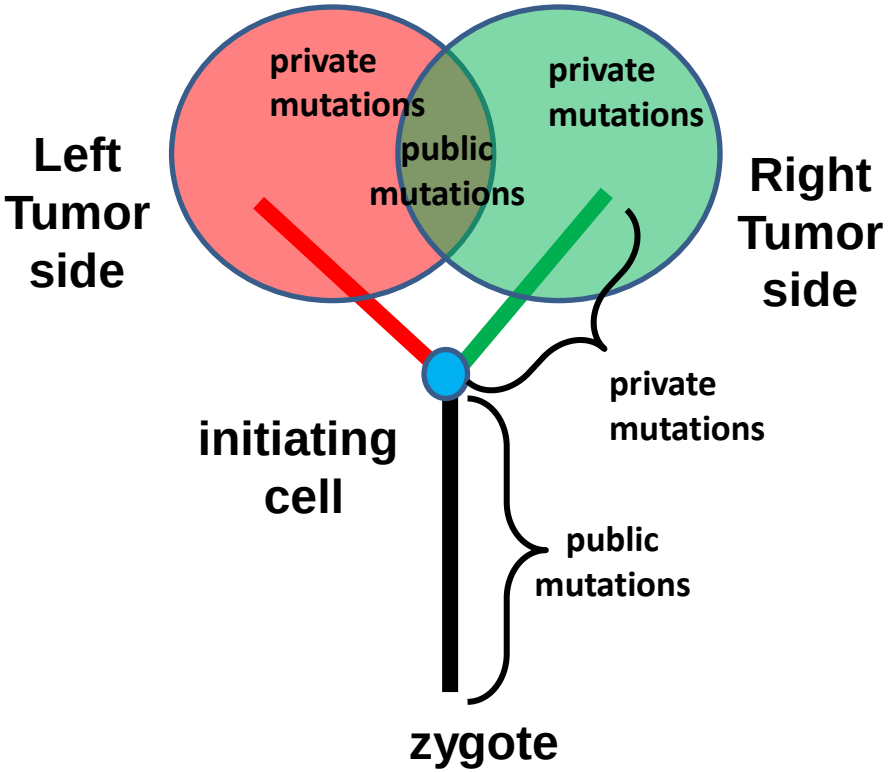


3) Something In Between

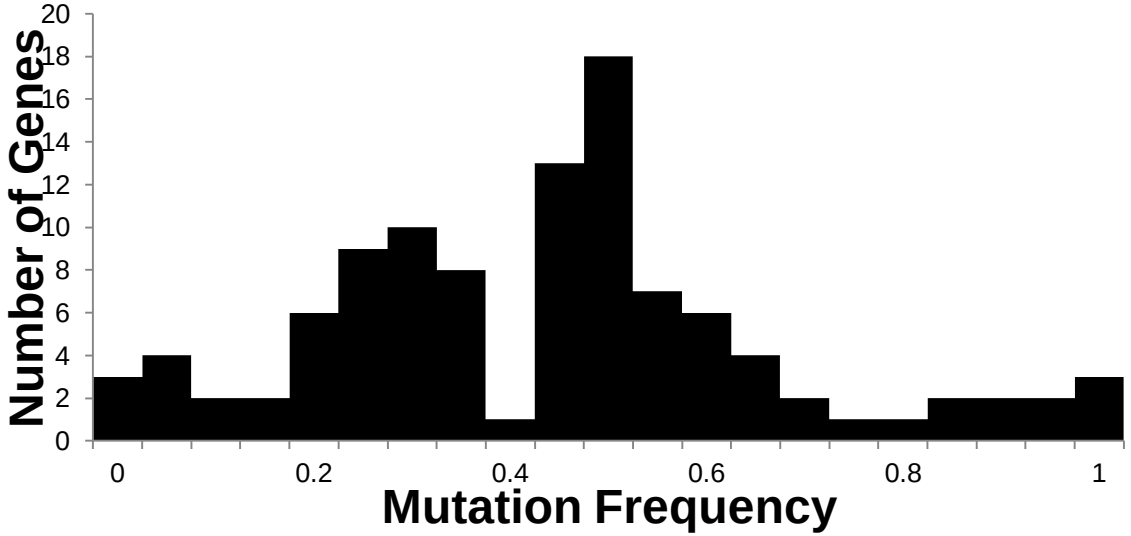
Experimental Approach

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)

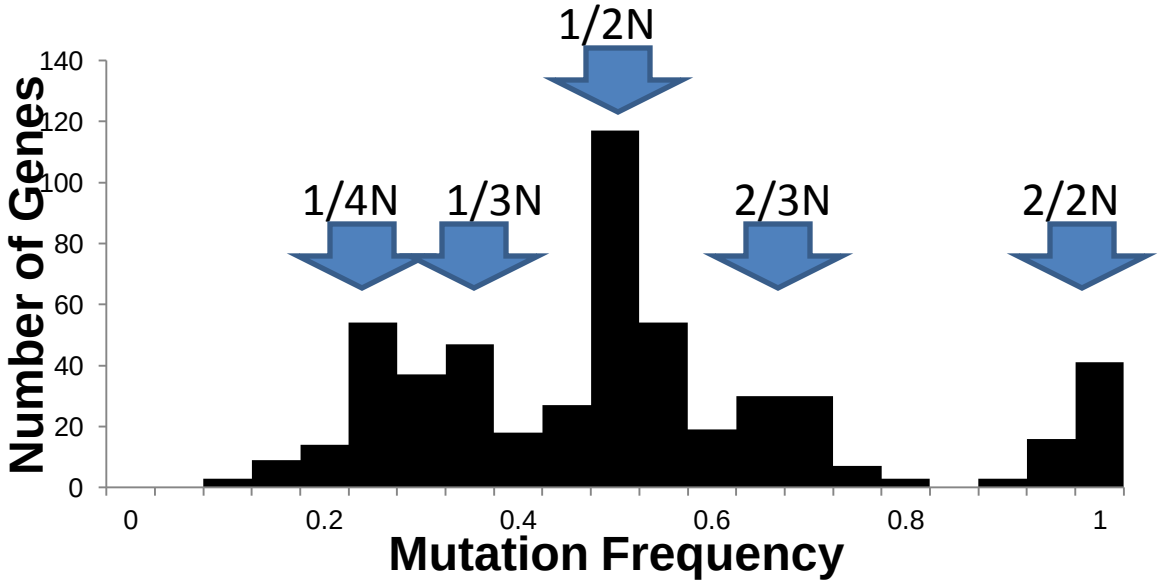




Bulk Resequencing Data: Continuous Mutation Frequencies



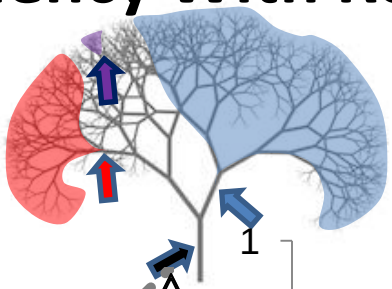
Gland Resequencing Data: "Quantum" Mutation Frequencies



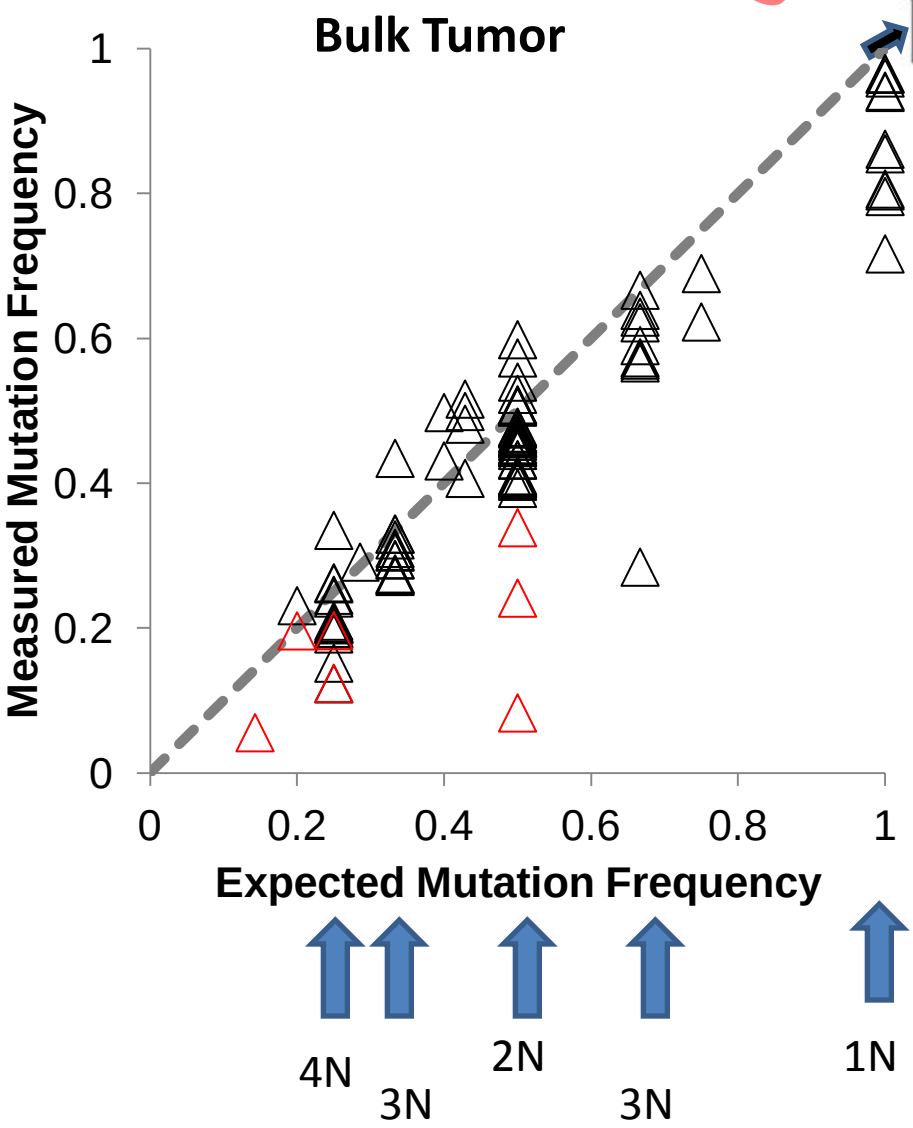
Mutation Frequency With Respect To Ploidy

BLACK Symbols = Public Mutations

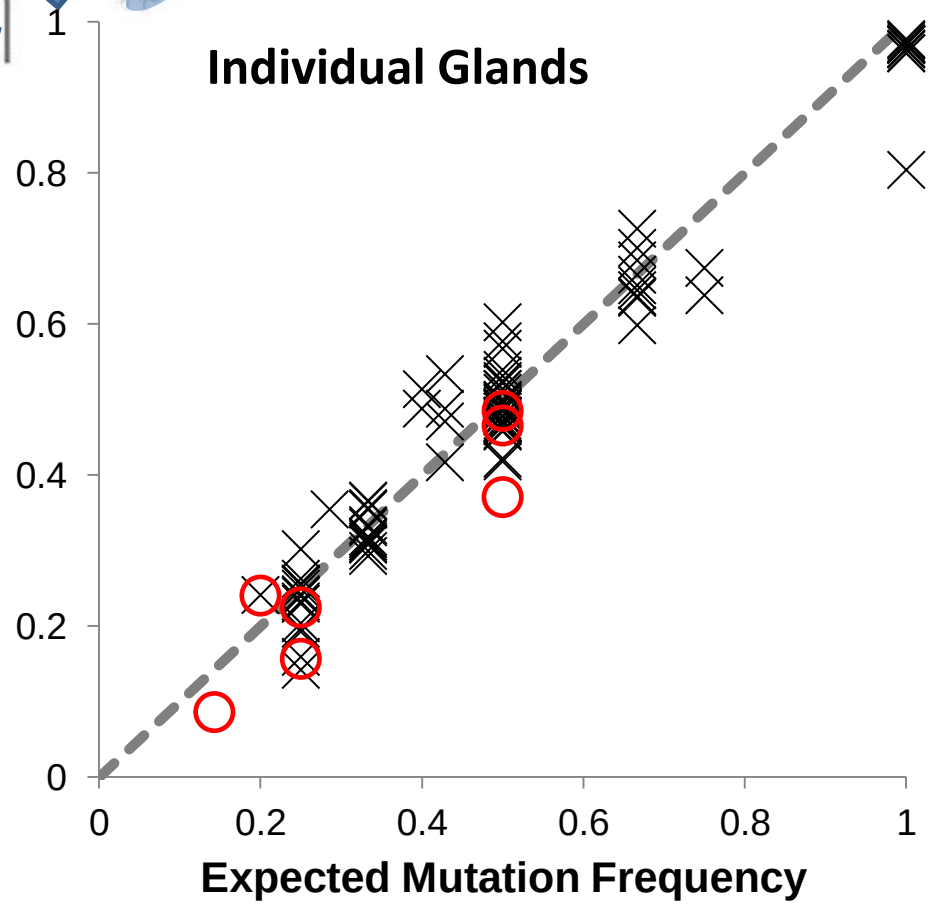
RED Symbols =Private Mutations



Bulk Tumor



Individual Glands



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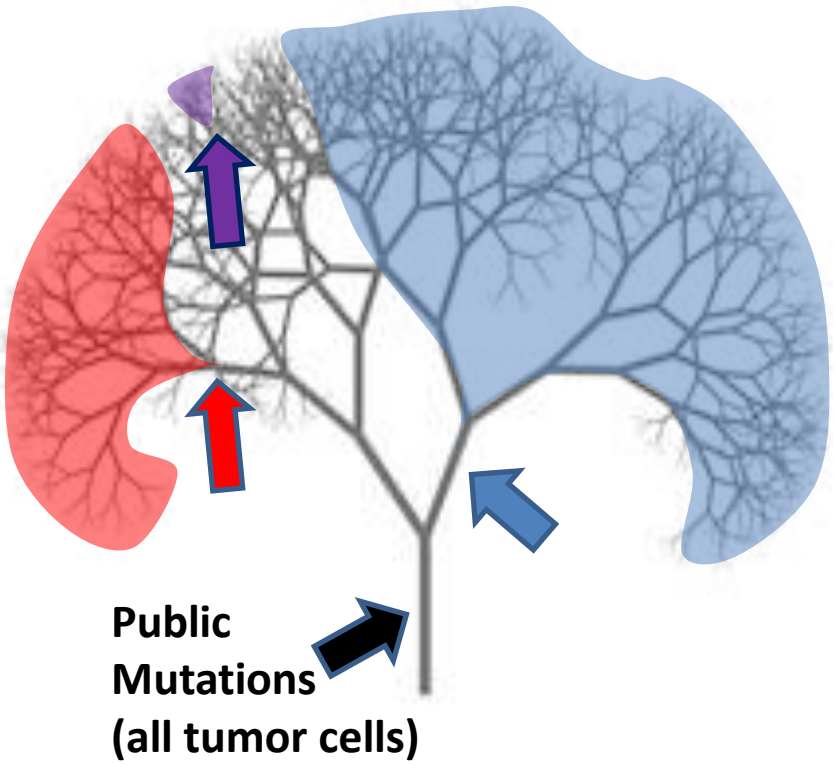
3) SNP Microarray: Many Average Gland CNAs Are “Quantum”
4) Mutation Resequencing: “Quantum” or “Fixed” Point Mutation Frequencies



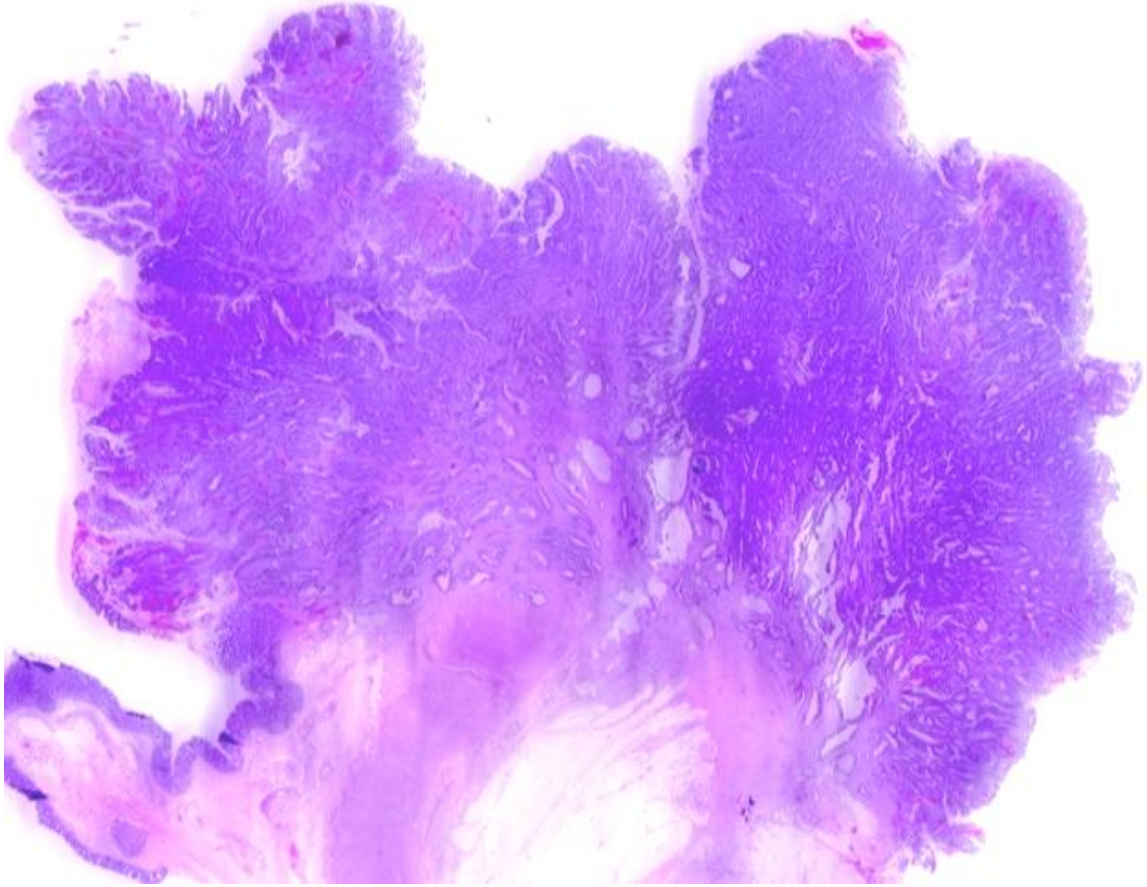
Model System: Human Colorectal Cancer

Specific Goal:

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

Clinical Question:

Are Tumors “Born To Be Bad”?



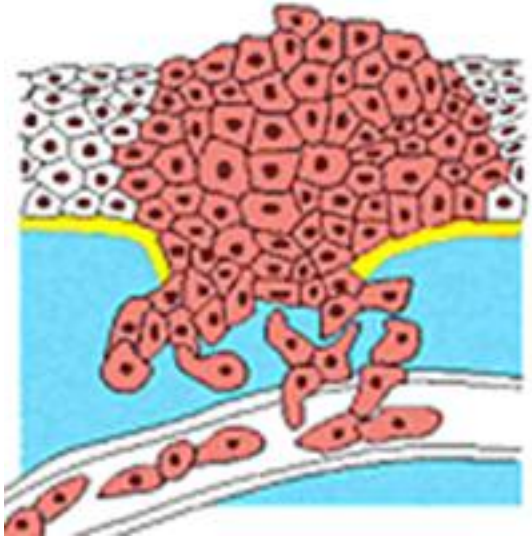
“Born To Be Bad”

What is “Bad” Clinically?: Death

How Do Tumors Kill?

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

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

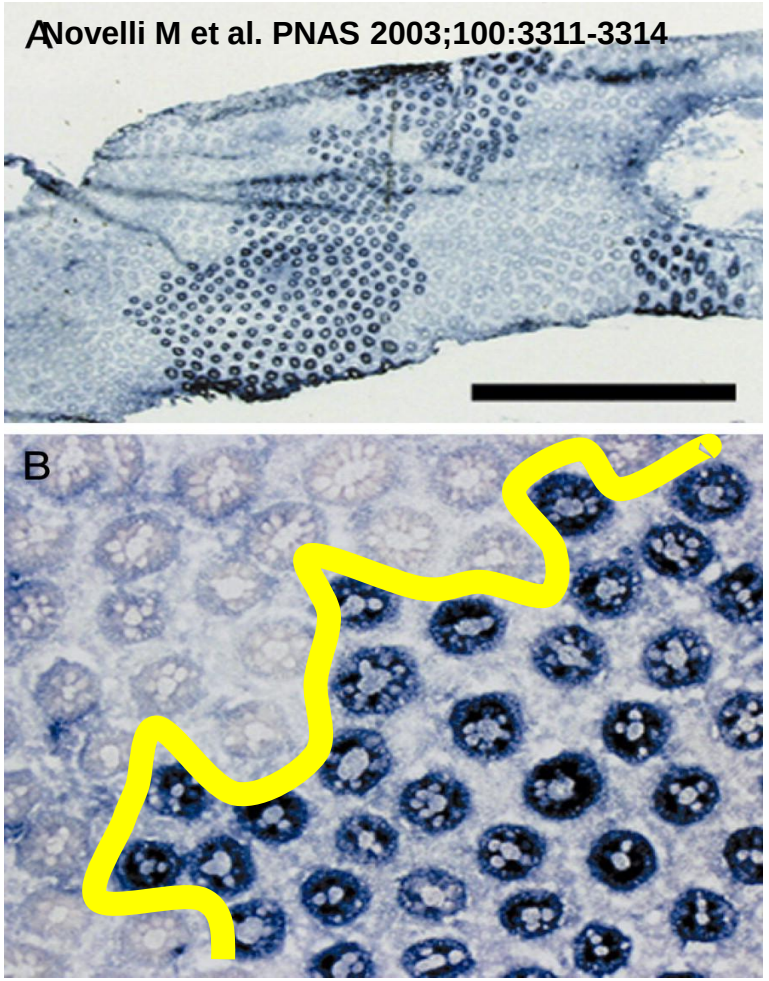


"Born To Be Good"

Cell Proliferation And Movement Is Normal
But Cell Intermixing Is Abnormal

Development:

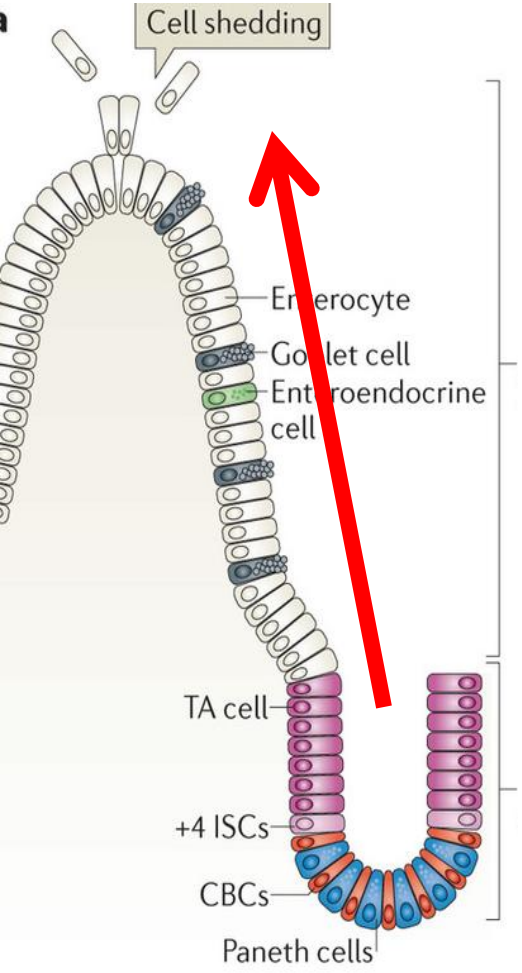
Clonal Patches

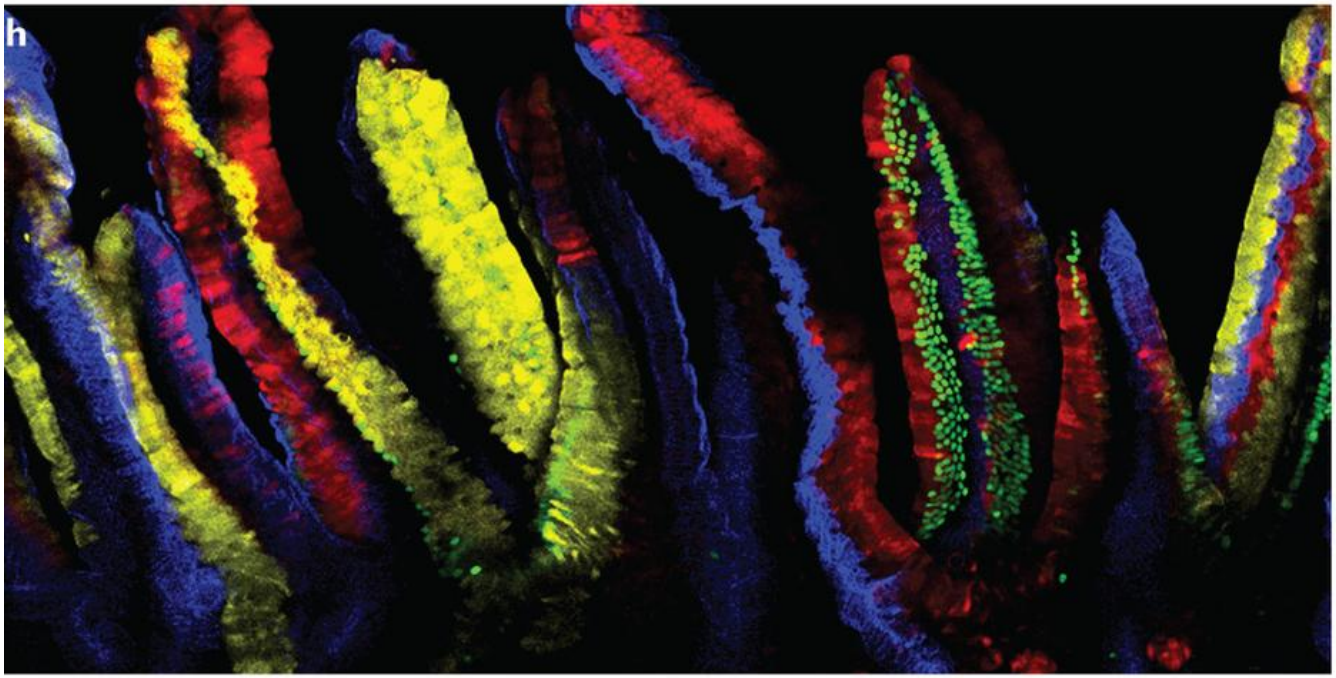


G6PDH expression: X-linked inactivation

“Born To Be Good”

Cell Proliferation And Movement Is Normal
But Cell Intermixing Is Abnormal

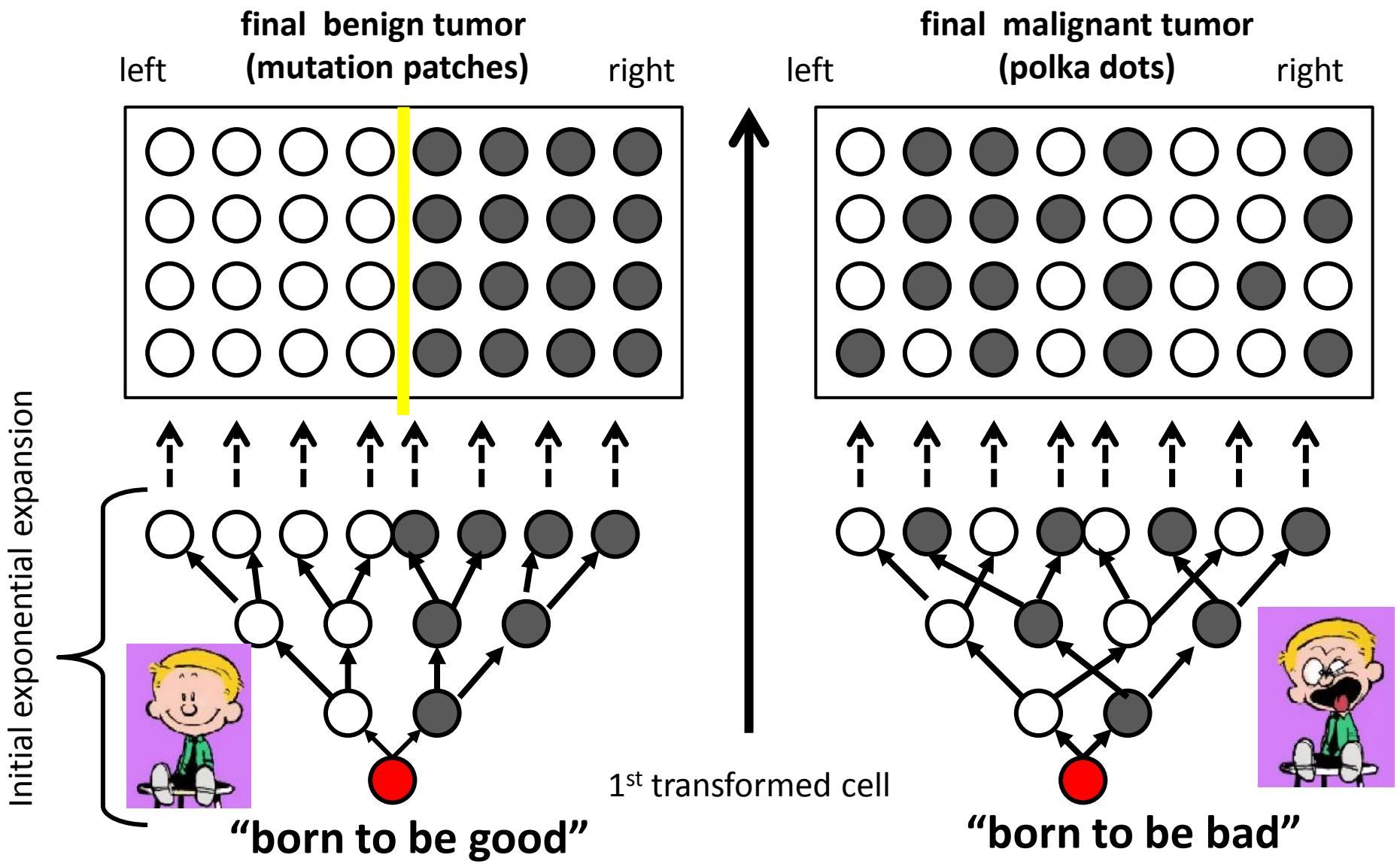




Intestinal Crypts:
Cell Migration in Orderly Columns

Born To Be Good/Bad

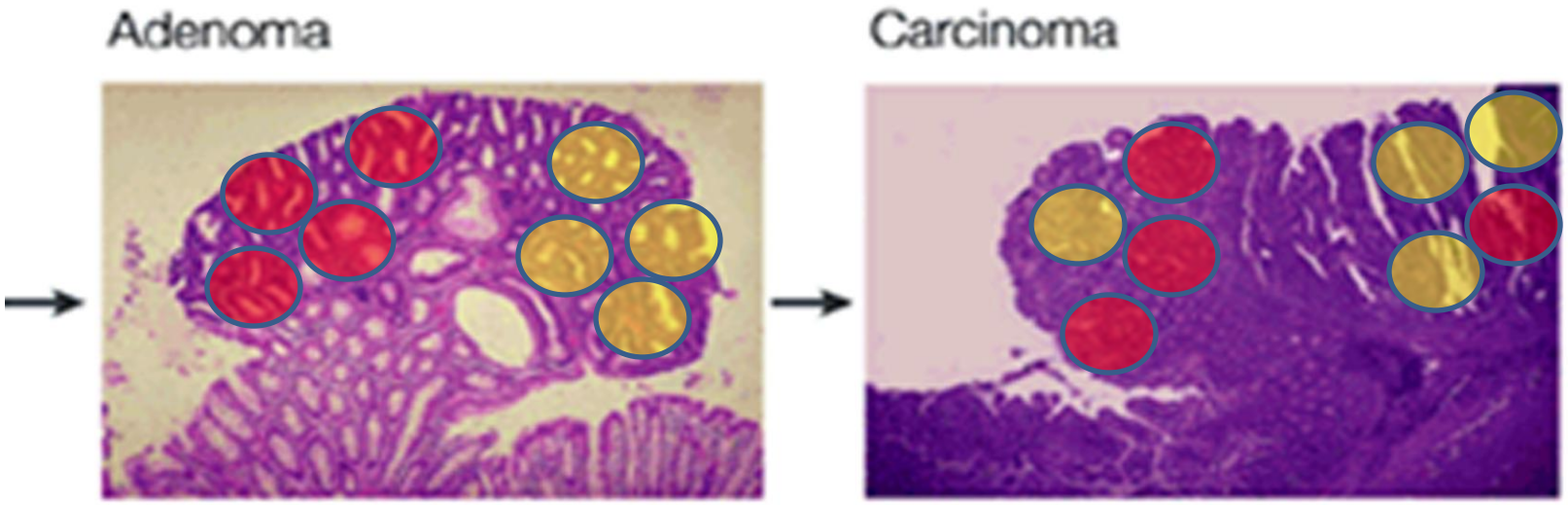
Effects of Early Cell Mixing



Colorectal Tumors

Benign Adenomas
(born to be good?)

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



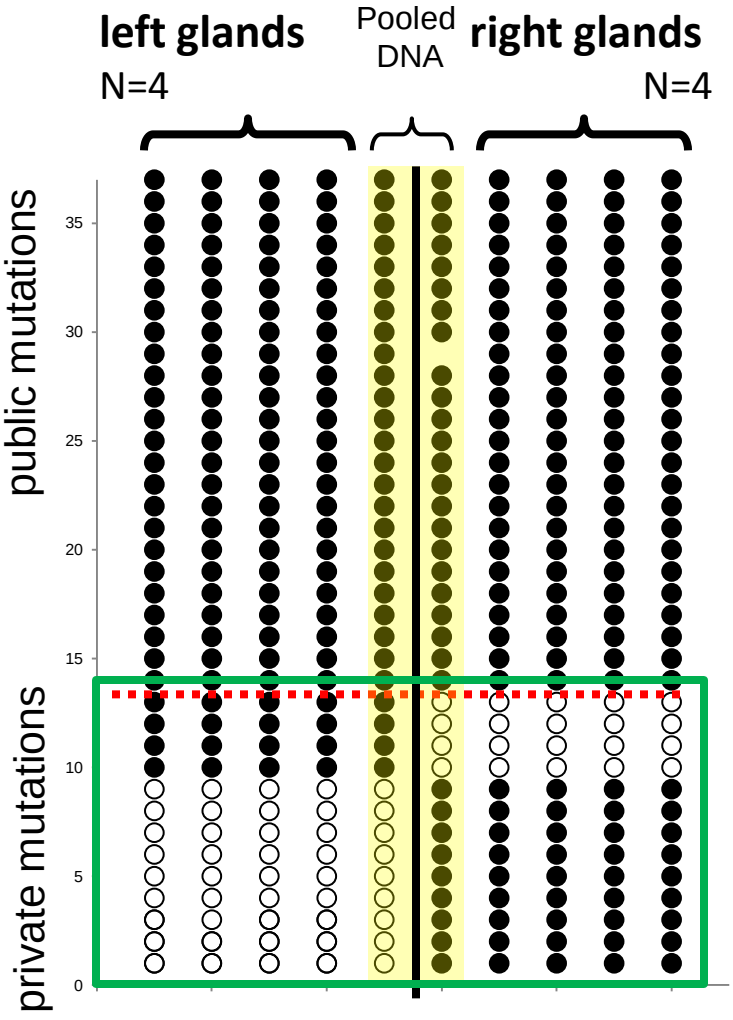
Mutation Patches

Mutation Polka Dots

Effects of Early Cell Mixing And Gland Mutation Fixation: "Identical" Glands On Opposite Tumor Sides

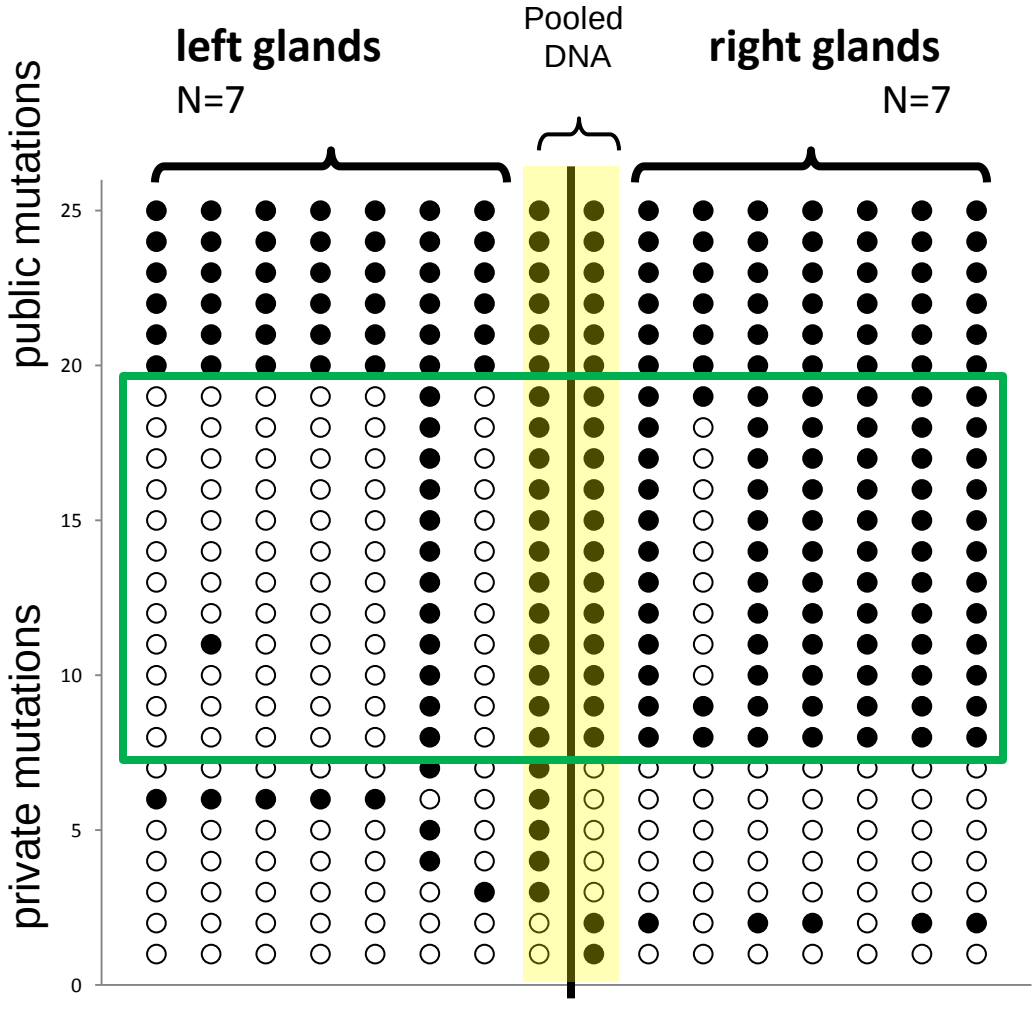
Point Mutations

ADENOMA



All Private Mutations
Side Specific

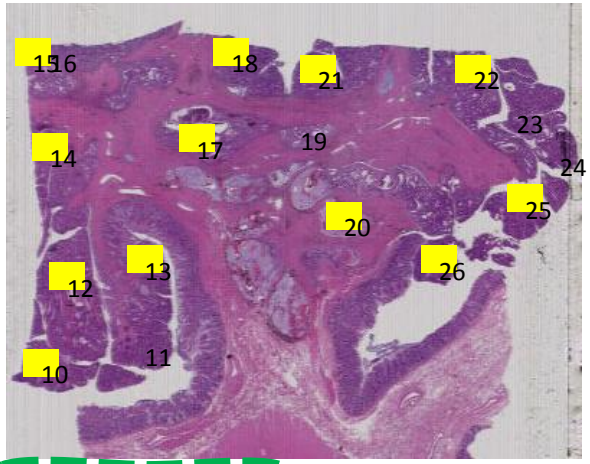
CARCINOMA

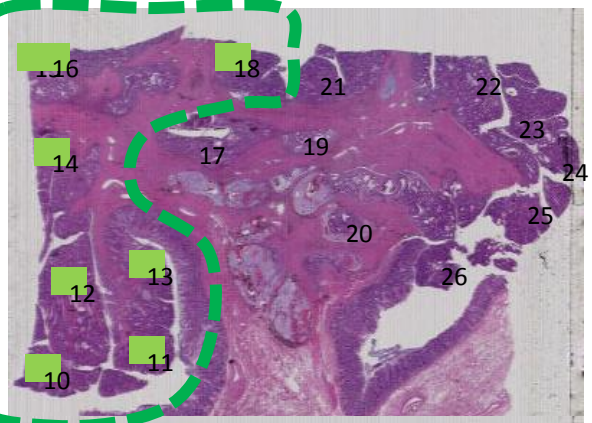


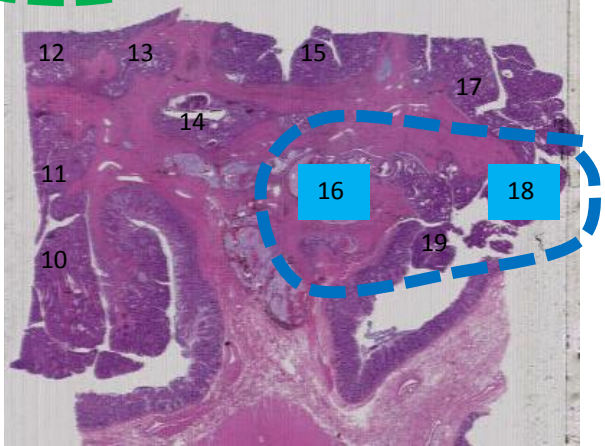
Some Private Mutations
Both Tumor Sides

Microdissection Data

Adenoma





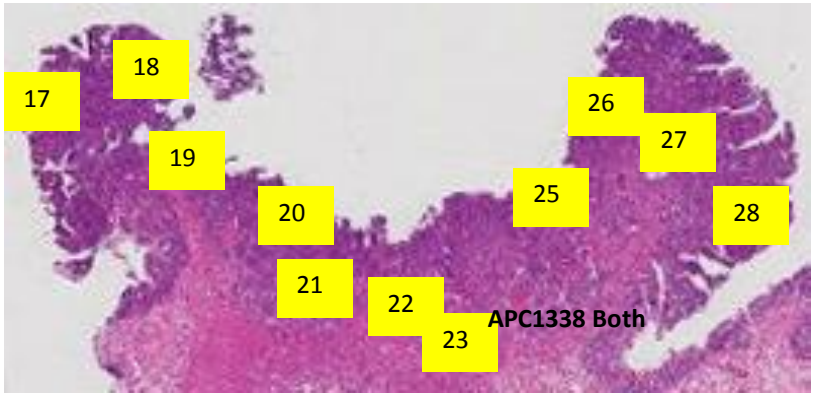


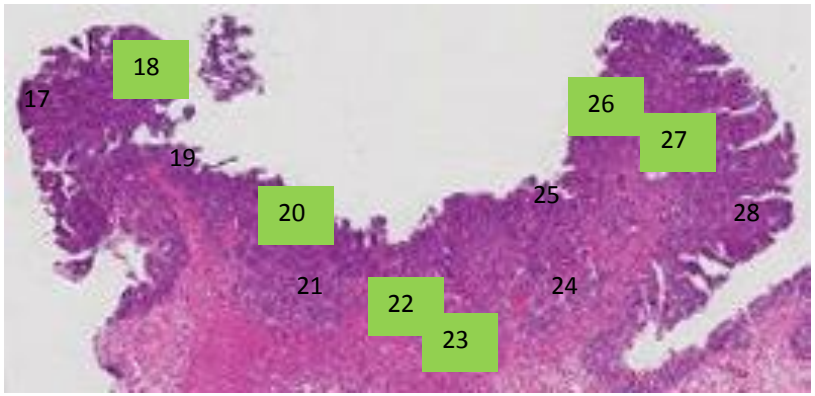
public mutation

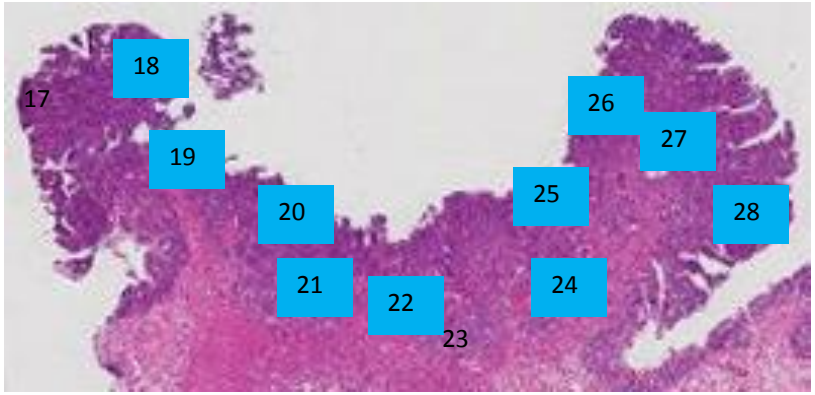
private mutation

private mutation

Cancer

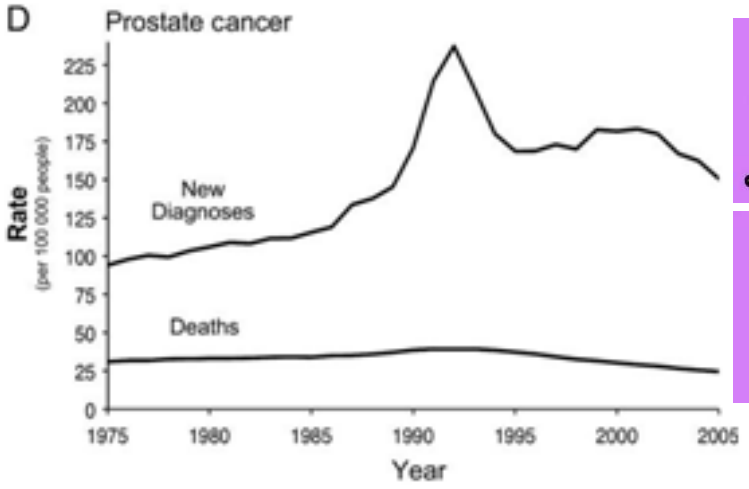




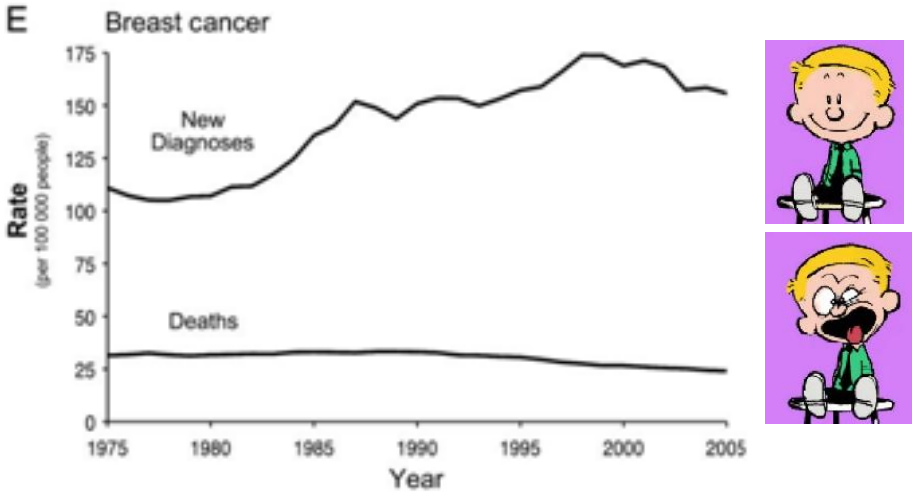


Difficult To Predict The Lethality Of Small Human Tumors

(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.

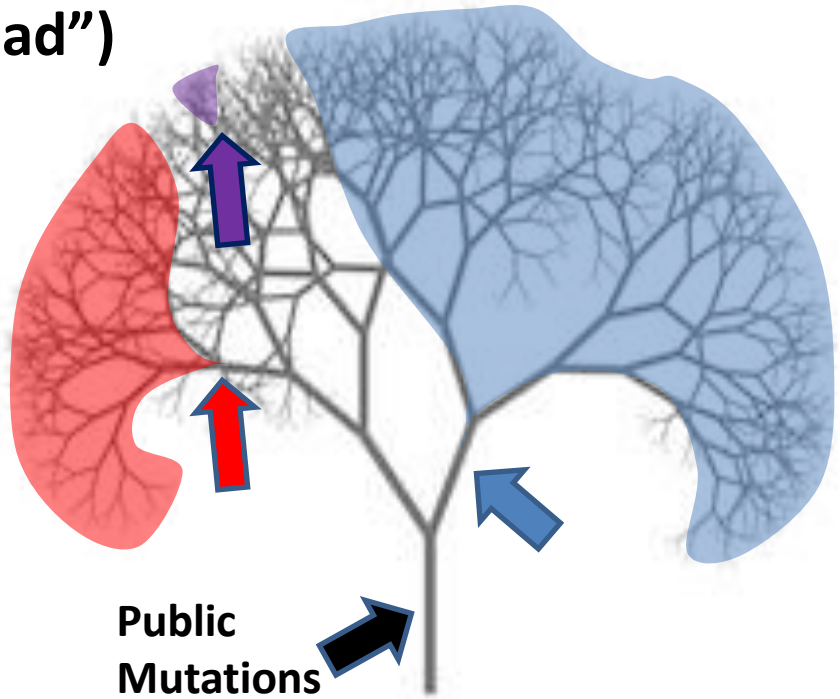
Welch H G , and Black W C JNCI J Natl Cancer Inst 2010;102:605-613

JNCI

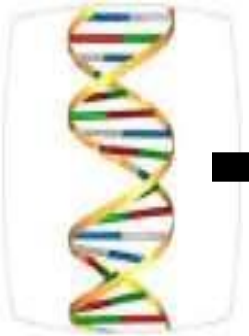
Summary of Tumor Gland Alterations

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" Point Mutation Frequencies
5) Mutation Location Informs Early Cell Mobility ("Born To Be Bad")

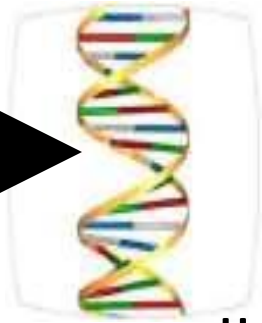
Single Clonal Expansion



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



zygote (start)



current cell (end)

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