

# Deciphering Renal Cancer Evolution

Charles Swanton MD PhD

BAUS 2014

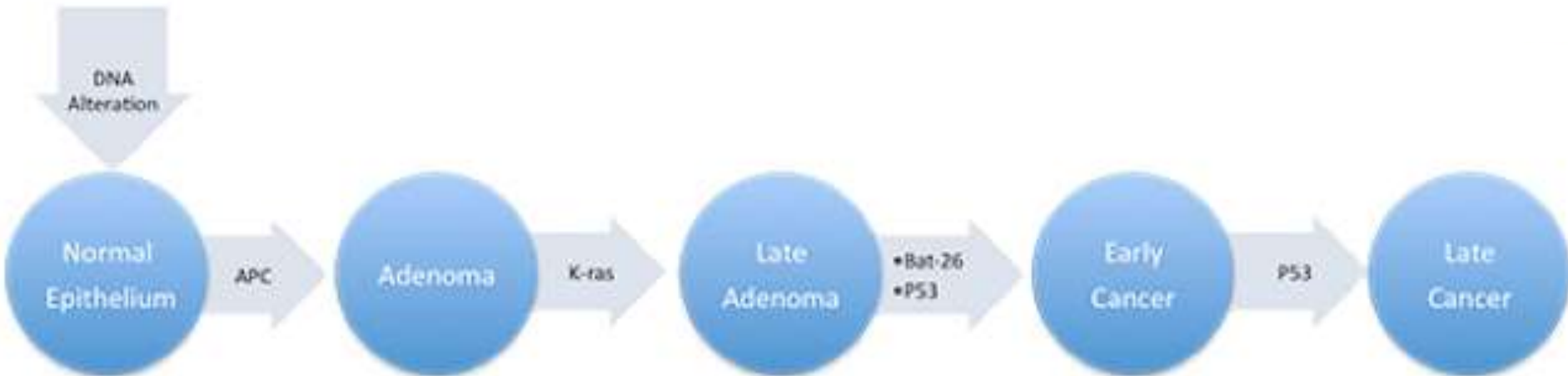
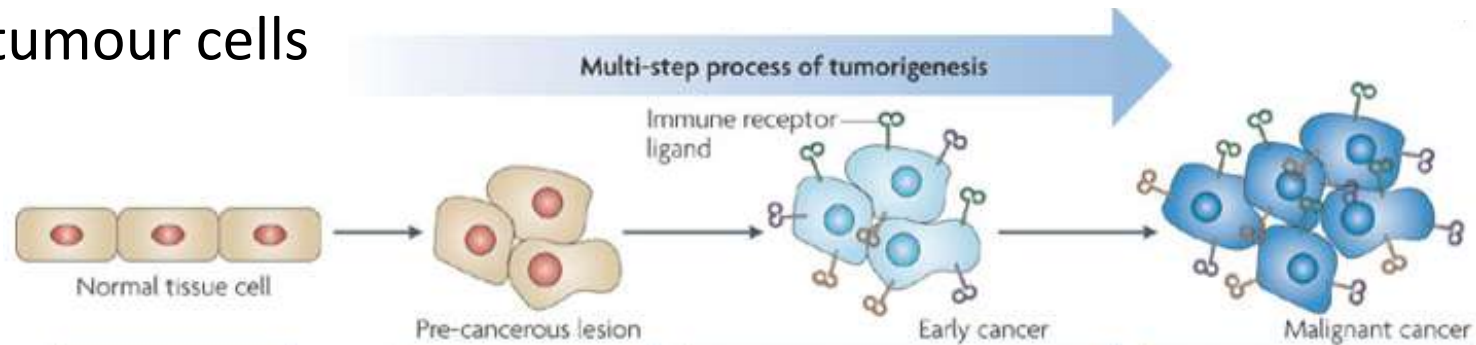
UCL Cancer Institute and

CR-UK Translational Cancer Therapeutics Laboratory



# Is Cancer a clonal disease evolving in a linear fashion ?

- Clonal succession and selective sweeps driving expansion of identical tumour cells

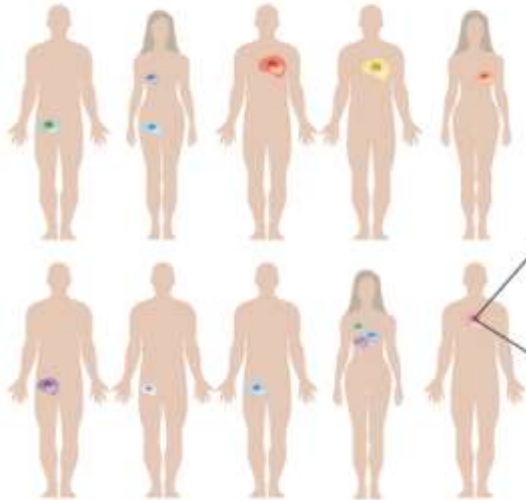


Nature Reviews | Immunology

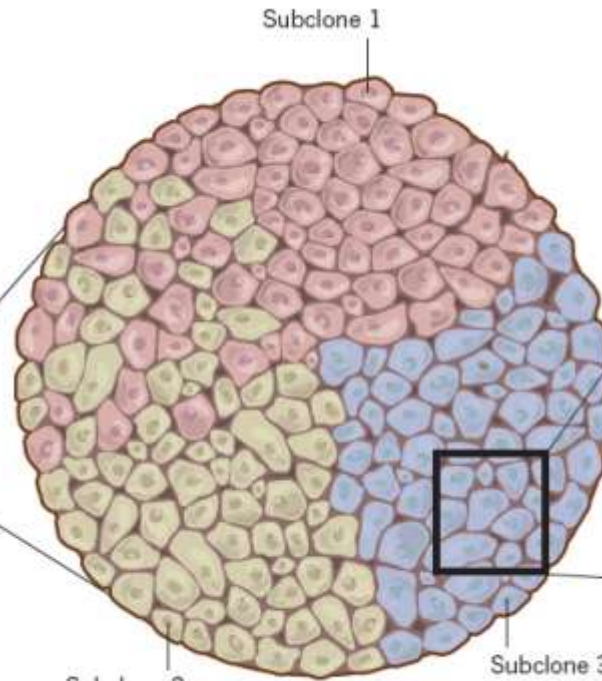
Curing a tumour composed of **identical** cells ought to be achievable.....

# Implications for Therapy and Outcome

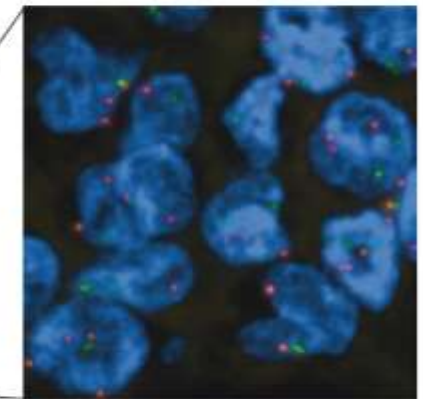
## Intertumour Heterogeneity



## Intratour Heterogeneity



## Intercellular Heterogeneity

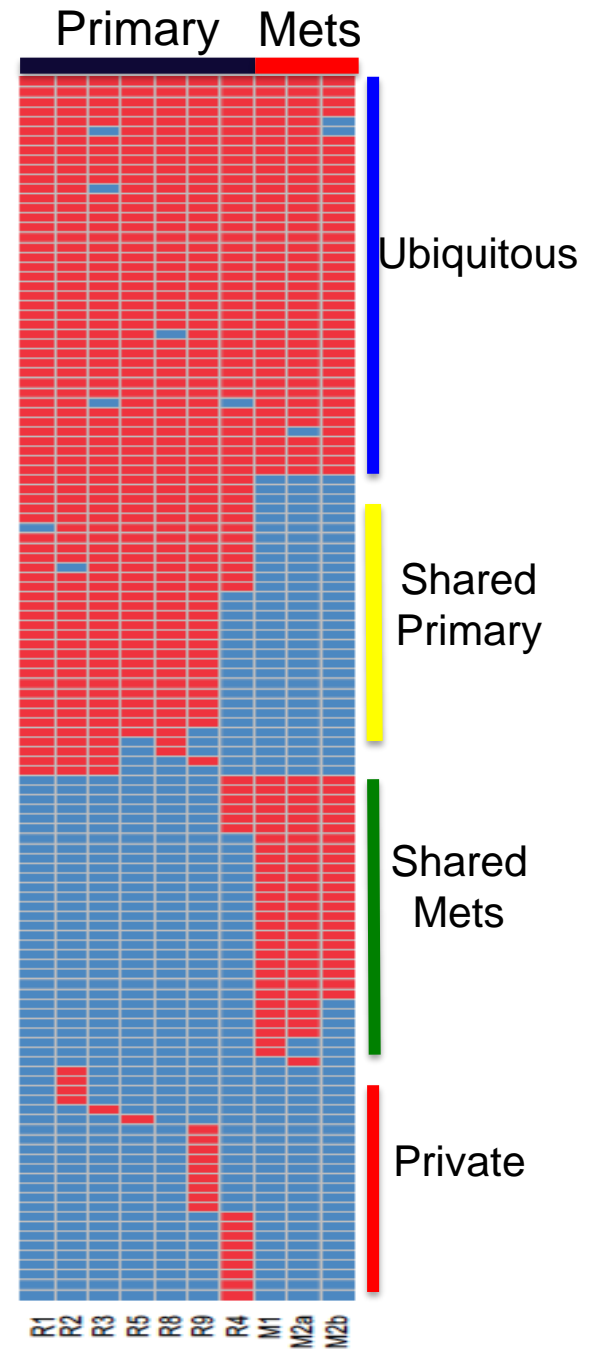
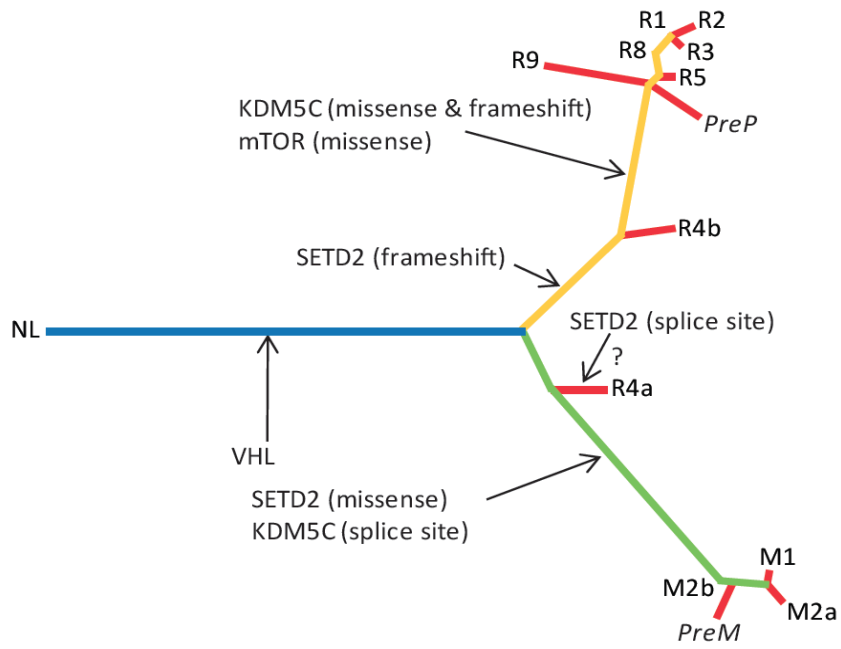
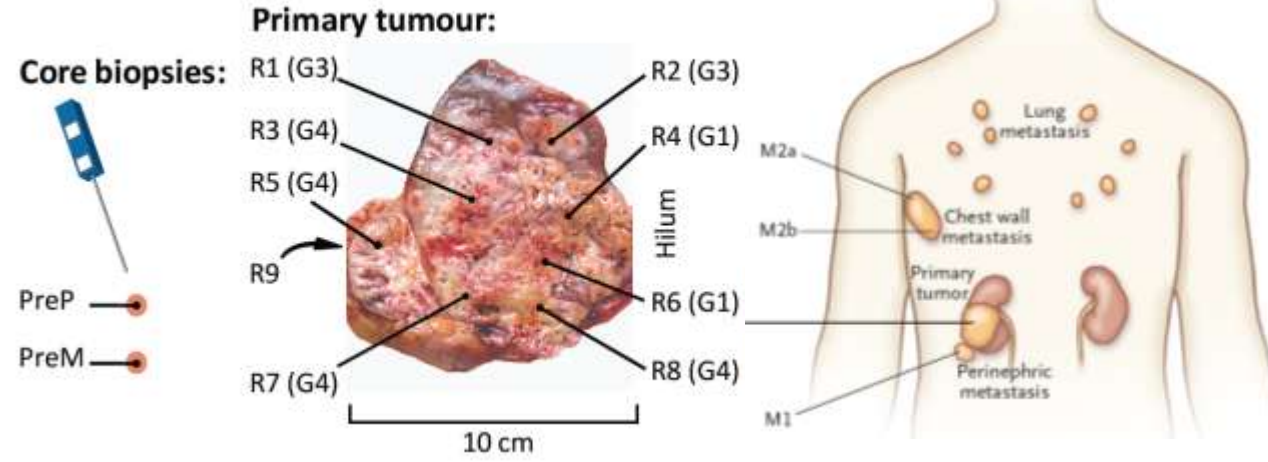


Intercellular genetic and non-genetic heterogeneity

Clonal heterogeneity

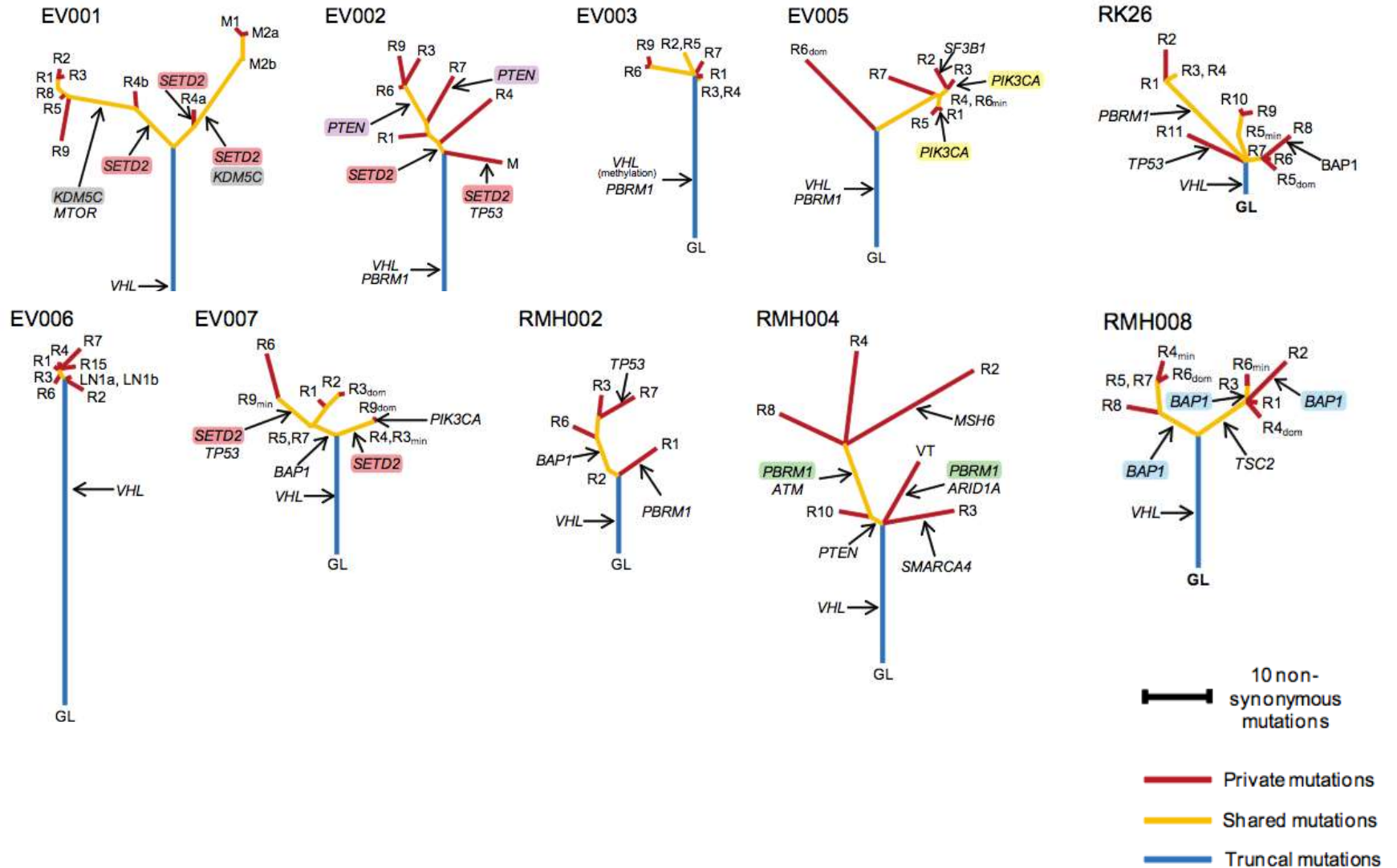
- Achieving cures in metastatic disease
- Cost of cancer drug development
- Cancer biomarker validation

# Branched Evolution in ccRCC



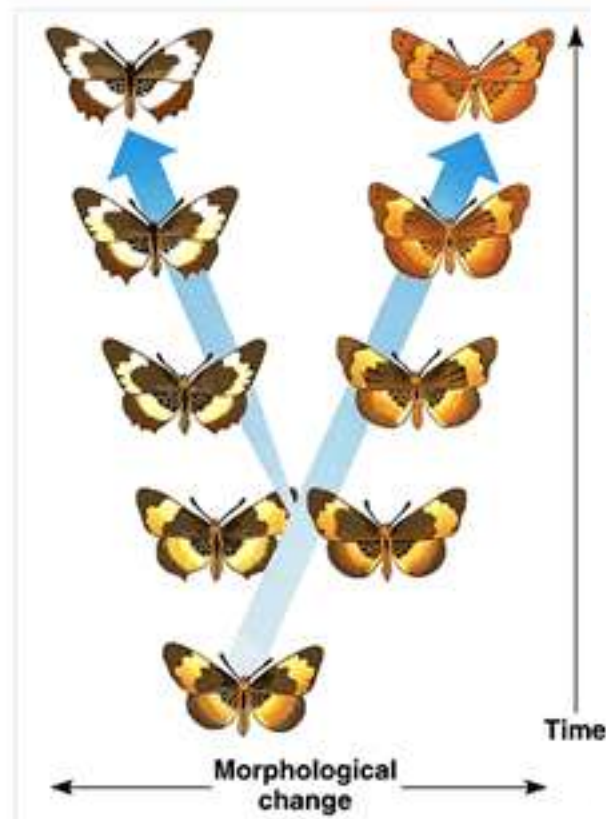
65% mutations are neterogeneous and not present in every biopsy

# Branched Evolution in ccRCC



# Microevolution: Gradualism

- Darwin argued that nature never makes jumps : *natura non facit saltum*
- Profound change is the result of a slow but continuous processes
- Gradual accumulation of small mutations as drivers of change (Neo-Darwinism)




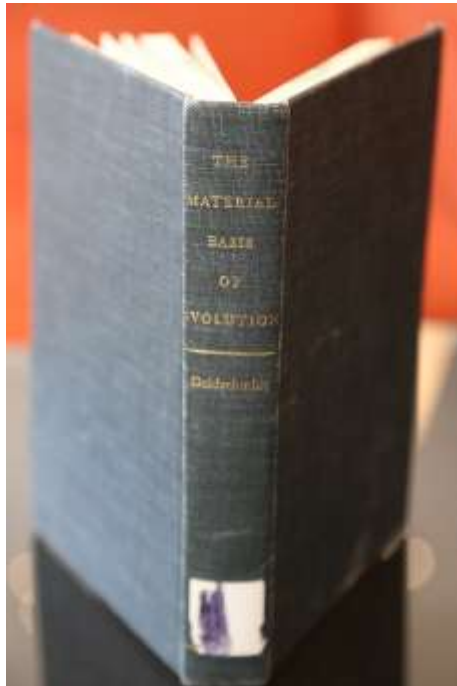
# Challenge

## Cancer Macroevoolution



# Macroevolution: “Hopeful Monsters”

- Goldschmidt argued that large changes in evolution were caused by “macromutations”
- Chromosomal rearrangements result in **Macroevolutionary leaps**  **Speciation**
- **Rare events resulting in profound change: “Hopeful monsters”**

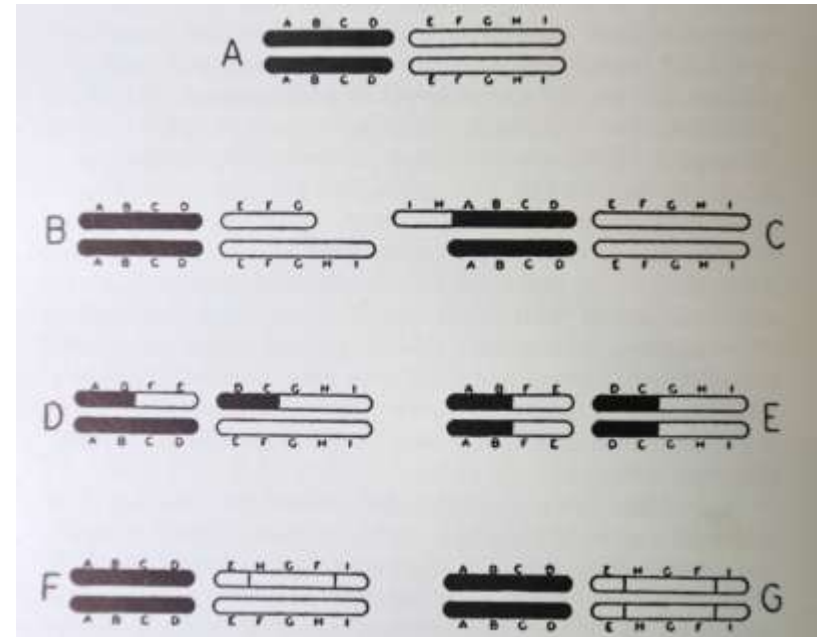




# Macroevolution and Hopeful Monsters

“**macroevolution** must proceed by a different genetic method.... Only the **arrangement of the serial chemical constituents of the chromosome into a new, spatially different order**; ie. A new chromosomal pattern, is involved”.

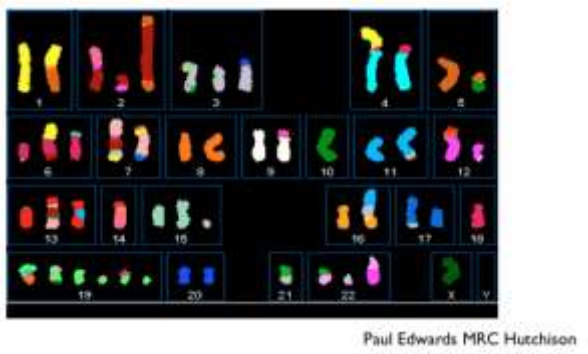
**Goldschmidt “Material Basis of Evolution” 1960**



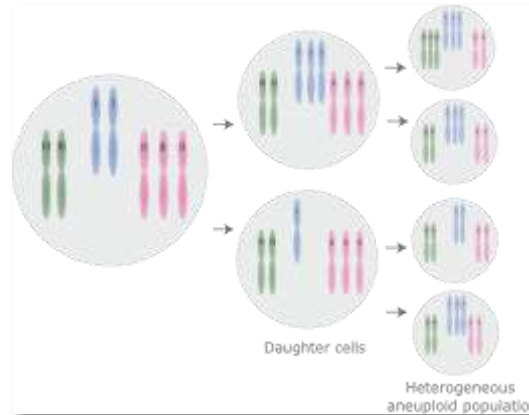
*Fig 35 Simple types of chromosomal rearrangements*

# Patterns of Cancer Chromosomal Rearrangements

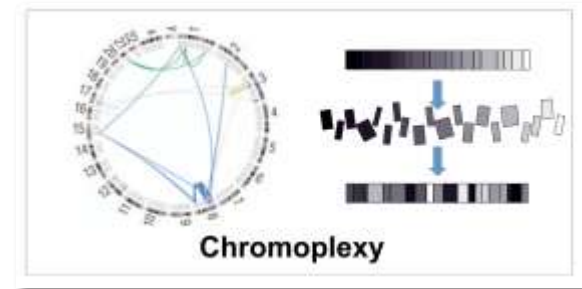
## Structural CIN



## Numerical CIN

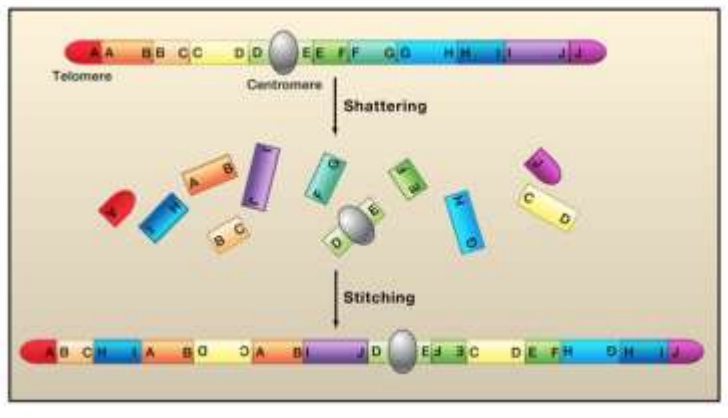


## Chromoplexy (Garraway)

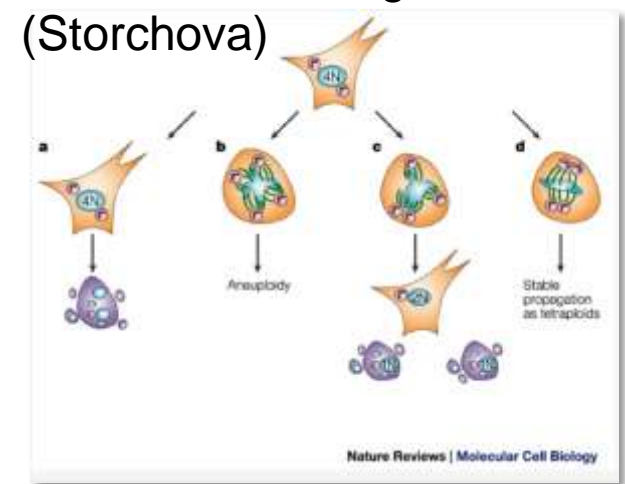


## Chromothripsis (Campbell/Meyerson)

Single chromosome fragmented and reassembled



## Genome Doubling (Storchova)



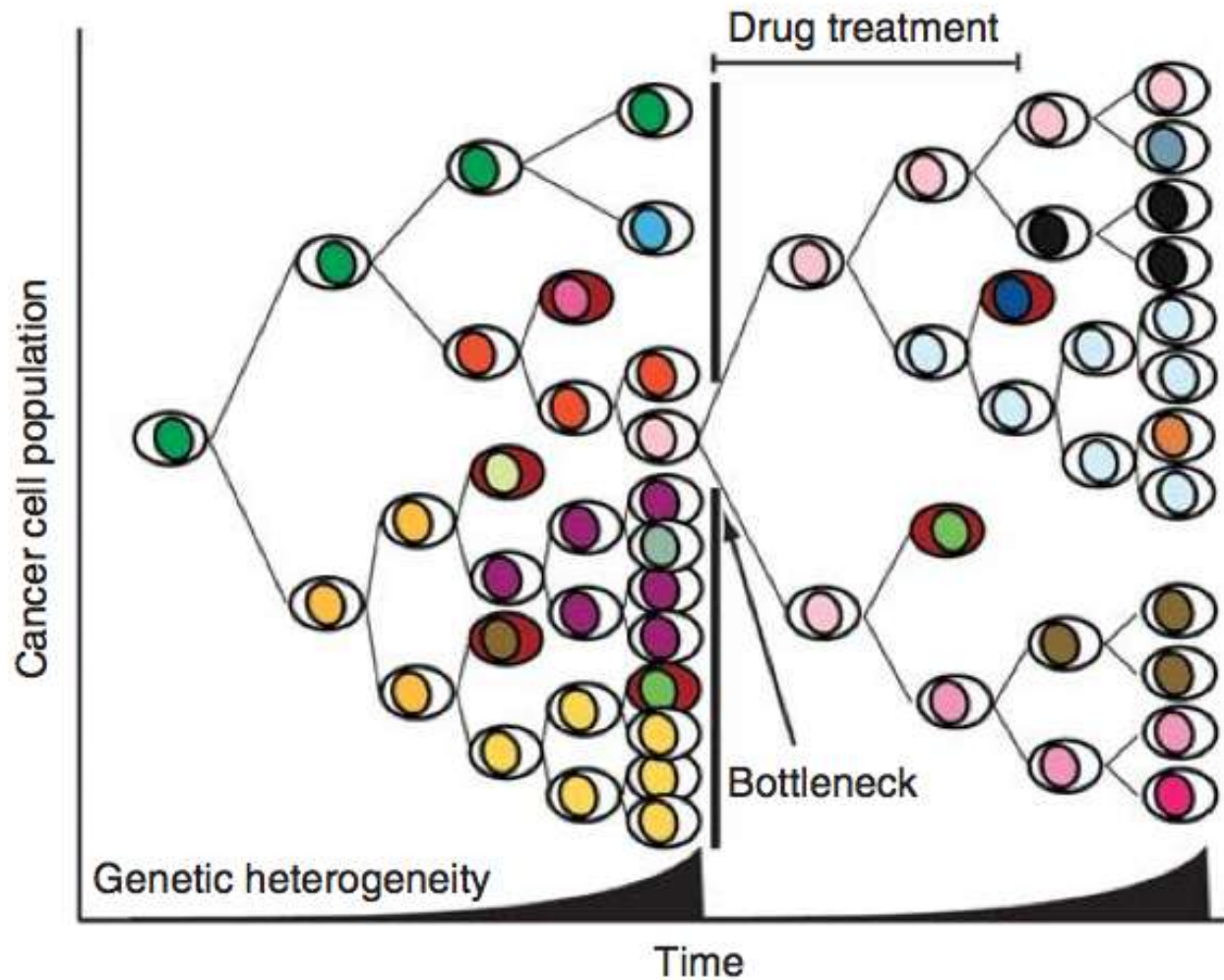
Generates Profound Cell-to-Cell heterogeneity: fuel for phenotypic change (Pavelka Nature 2010)

# Intra-metastatic Heterogeneity Driven by Chromosomal Instability



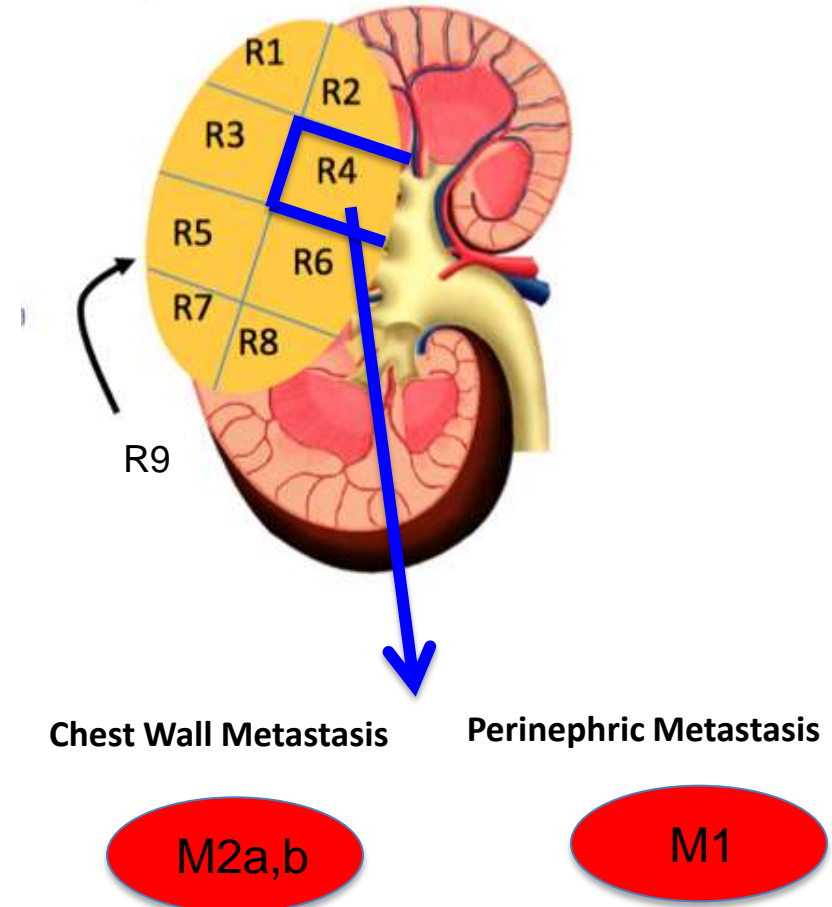
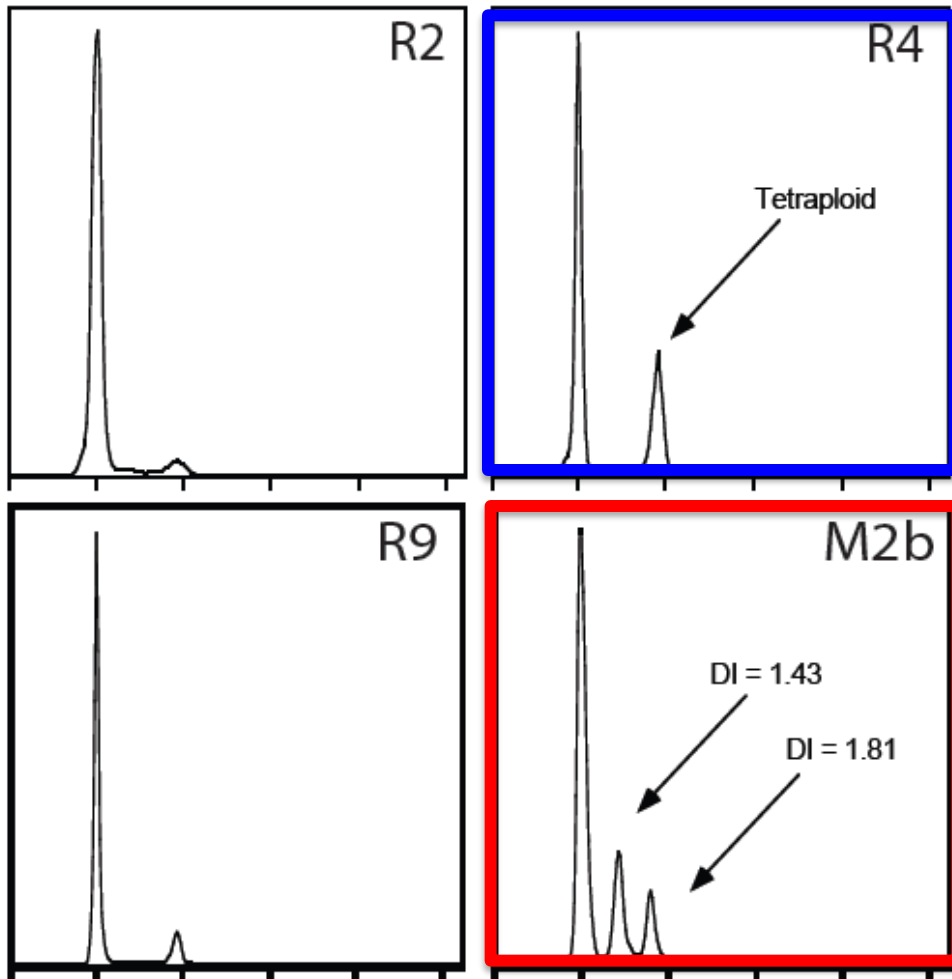
# Bottlenecking at Metastatic Sites

## Is there a Substitute for Diversity ?

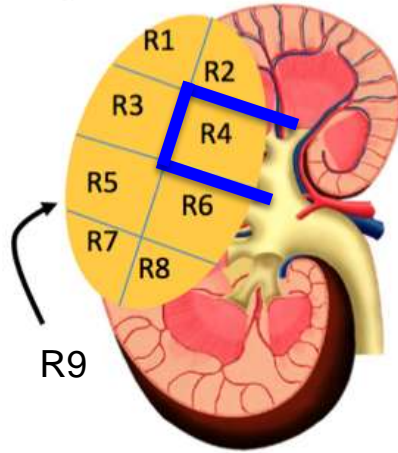
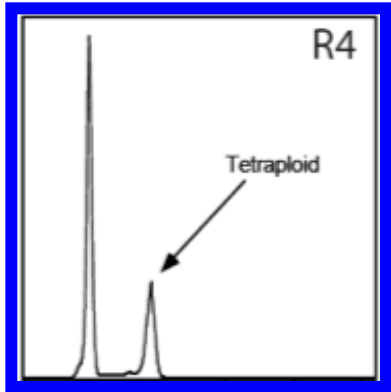


# Ploidy Analysis of Patient 001 Nephrectomy

Tetraploid intermediate in Region 4:  
Aneuploid progeny at metastatic sites



# Allelic Imbalance

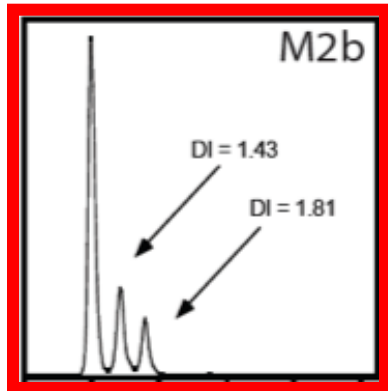
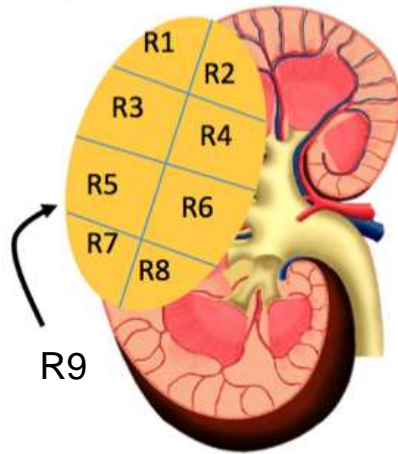


**Region 4 has Doubled its Genome**



**Chromosome**

# Intra-Metastatic Tumour Heterogeneity driven by Chromosomal Instability

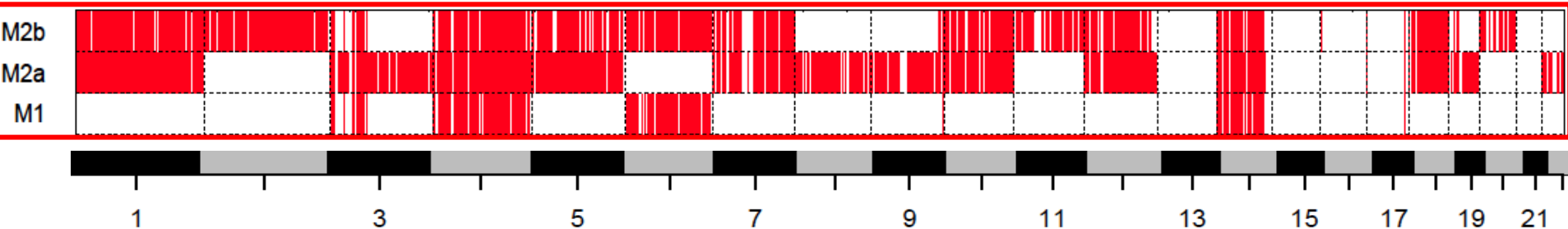


Chest Wall Metastasis

M2a,b

Perinephric Metastasis

M1



# Intra-tumour heterogeneity Chromosomal Instability and Poor Clinical Outcome

Cancer Type	Method of measuring CIN	CIN associated with	Reference
<b>Lung cancer (NSCLC)</b>	FISH (n=63)	Poor prognosis (OS & DFS)	Choi et al. (2009)
	FISH (n=47)	Poor prognosis (OS)	Yoo et al. (2010)
	FISH (n=50)	Poor prognosis (OS)	Nakamura et al. (2003)
	12-gene genomic instability signature (n=647)	Poor prognosis (OS)	Mettu et al.(2010)
	CIN70 signature (n=62)	Poor clinical outcome	Carter et al. (2006)
<b>Breast cancer</b>	SSI (n=890)	Poor prognosis (OS)	Kronenwett et al. (2004)
	SNP (n=313)	Poor prognosis (MFS)	Smid et al. (2010)
	12-gene signature (n=469)	Poor prognosis (DFS & RFS)	Habermann et al.(2009)
	CIN70 signature (n=1866)	Poor clinical outcome	Carter et al. (2006)
	FISH (n=31)	Lymph-node metastasis and ER negativity	Takami et al.(2001)
<b>Myelodysplastic syndrome</b>	FISH (n=65)	Poor prognosis (DFS)	Heilig et al.(2010)
<b>Endocrine pancreatic tumors</b>	CGH (n=62)	Metastasis	Jonkers et al.(2005)
<b>Colon cancer</b>	12 gene genomic instability signature (n=92)	Recurrence	Mettu et al. (2009)
	Flow cytometry/ image cytometry (n = 10 126)	Poor prognosis	Walther et al.(2008)
<b>Ovarian cancer</b>	12-gene genomic instability signature (n=124)	Poor prognosis (RFS)	Mettu et al.(2010)
<b>Endometrial cancer</b>	SNP (n=31)	Poor prognosis (OS)	Murayama-Hosokawa et al. (2010)
<b>Synovial sarcoma</b>	CGH (n=22)	Poor prognosis (OS)	Nakagawa et al.(2006)
<b>Oral cancer (SCCs)</b>	FISH (n=77)	Poor prognosis (OS & DFS)	Sato et al.(2010)
	FISH (n=20)	(loco)regional tumour outgrowth	Bergshoeff et al. (2008)
<b>Diffuse Large B-cell Lymphoma</b>	Anaphase segregation errors (n=54)	Poor prognosis (RFS)	Bakhoum et al. (2011)

Abbreviations: NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; FISH, fluorescence in situ hybridization; SSI, stem line scatter index; CGH, comparative genome hybridisation; SNP, single-nucleotide polymorphisms; OS, overall survival; DFS, disease-free survival; MFS, metastasis-free survival; RFS, relapse free survival



# Opportunities for Clinical Trial Development

Define Actionable Mutations by Clonal Dominance



**Marco Gerlinger Stuart Horswell, James Larkin,  
Max Salm, Nik Matthews**



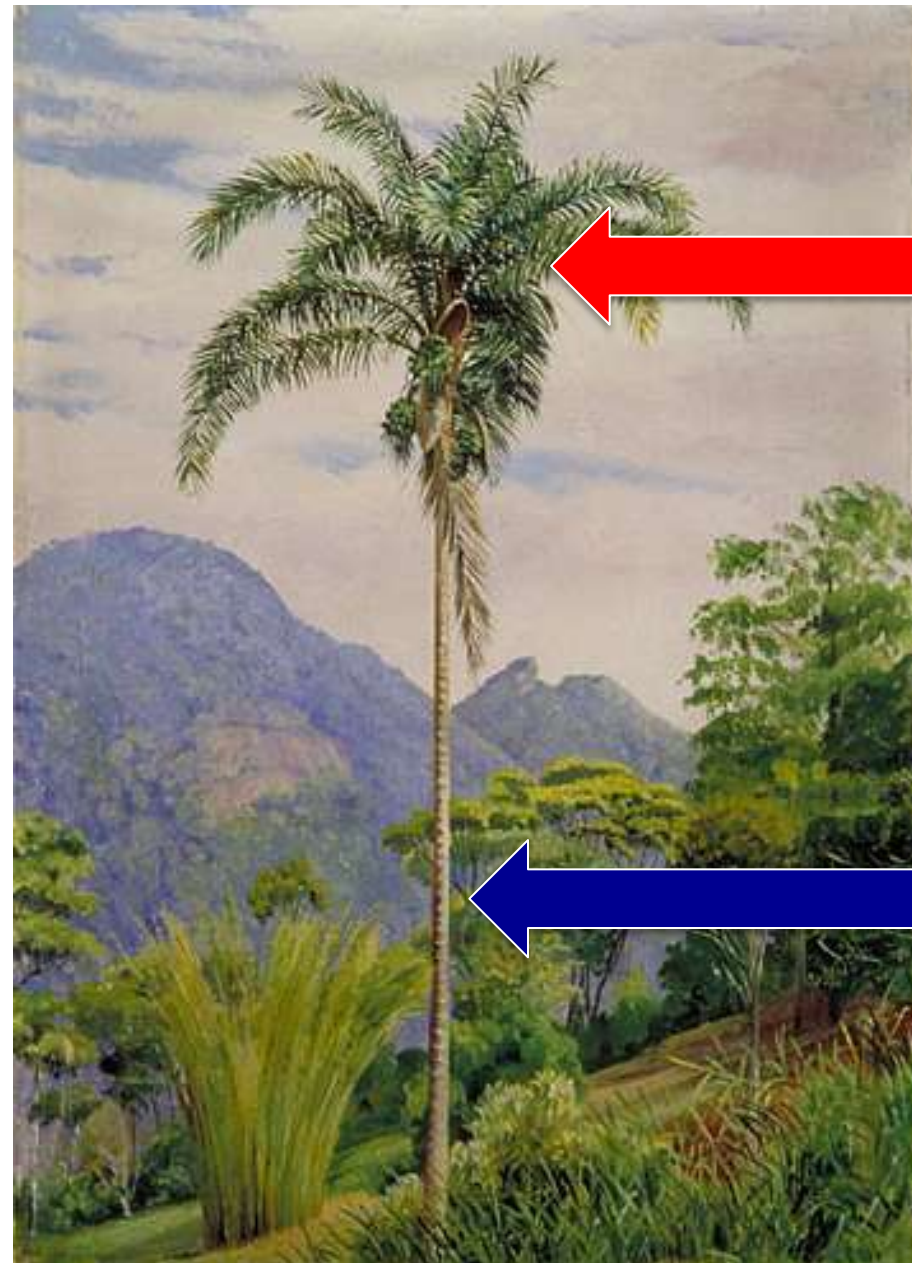
# Target Tumour Phylogenetic Trunks and Resolve Branches

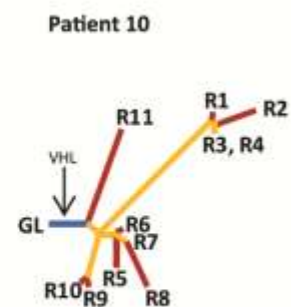
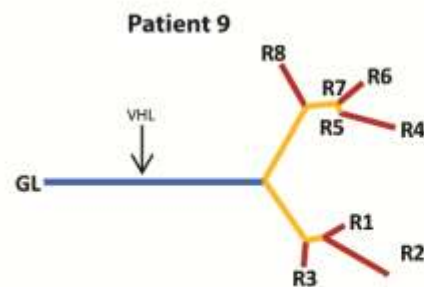
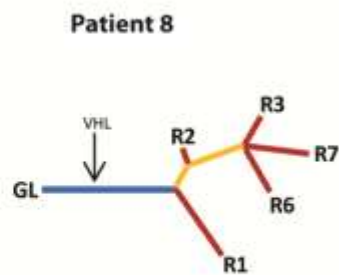
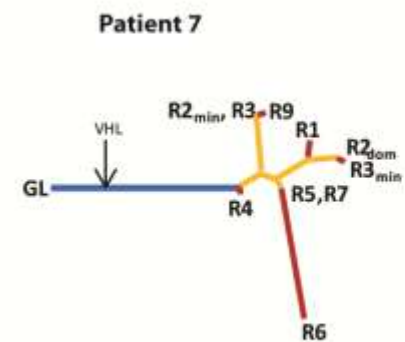
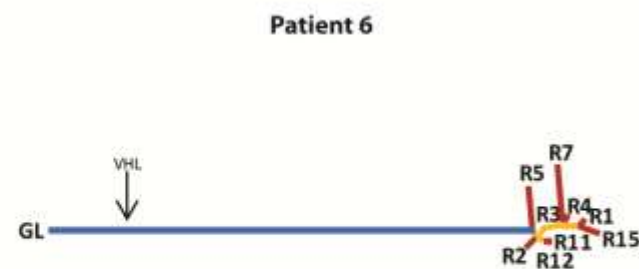
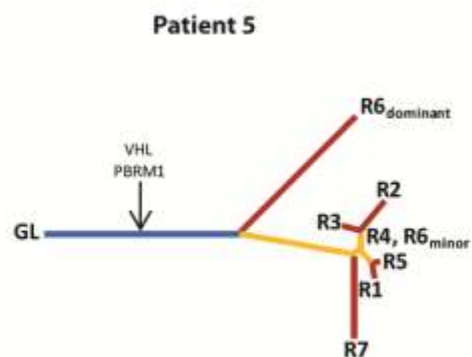
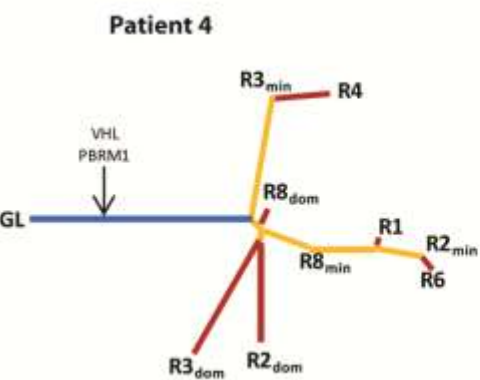
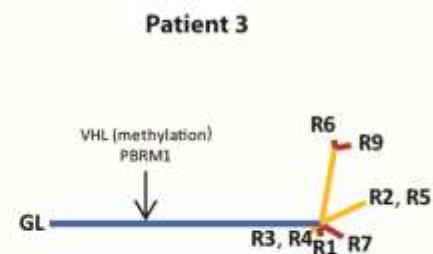
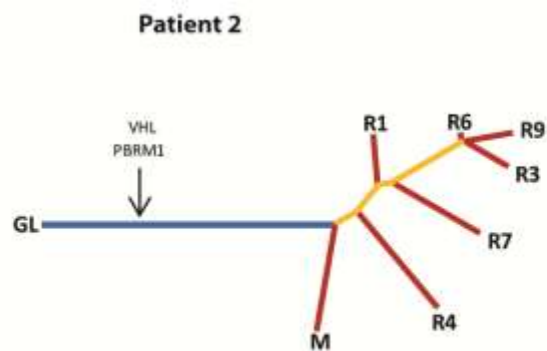
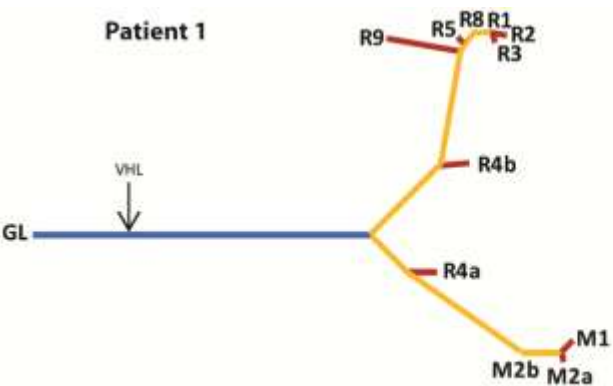
**Branched Genetic Events Present in  
Some Cancer Cells not others  
Dynamic during disease course**

**Monitor subclonal events to define  
drug resistance mechanisms**

**Trunk Genetic Events Present in  
Every Cancer Cell**

**DEFINE and TARGET TRUNK DRIVERS**

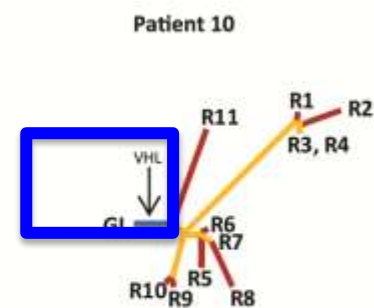
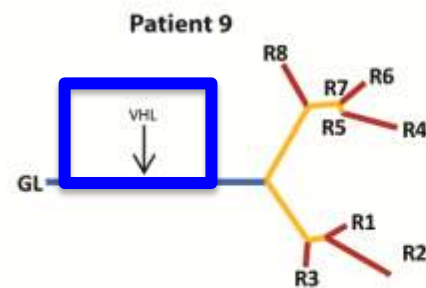
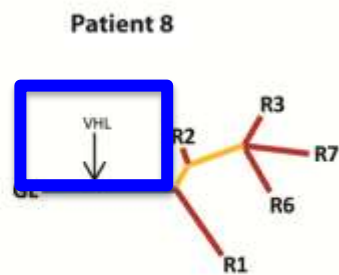
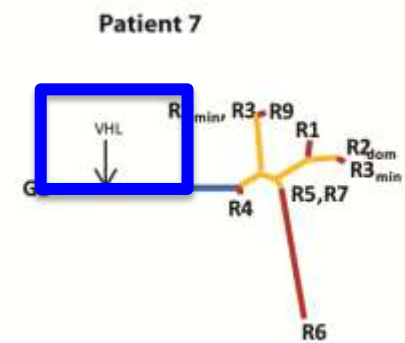
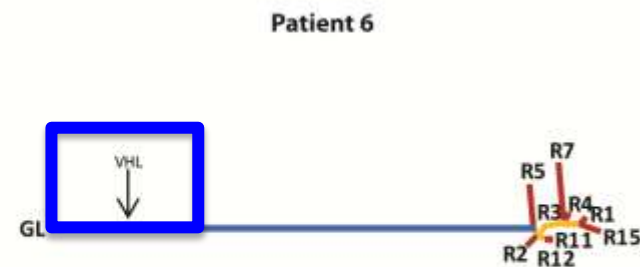
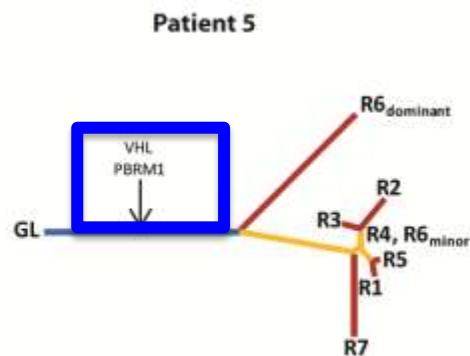
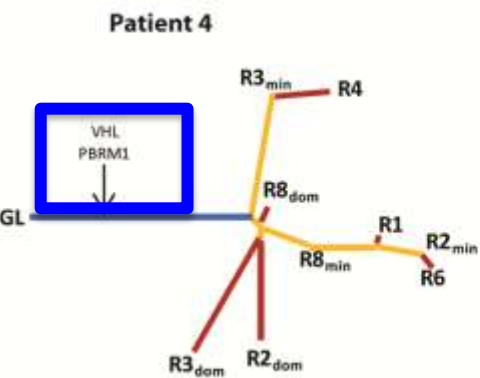
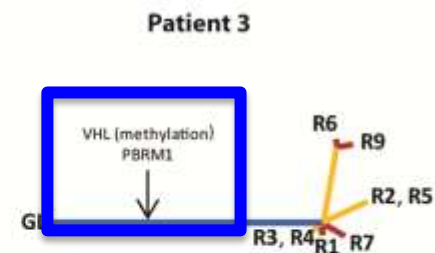
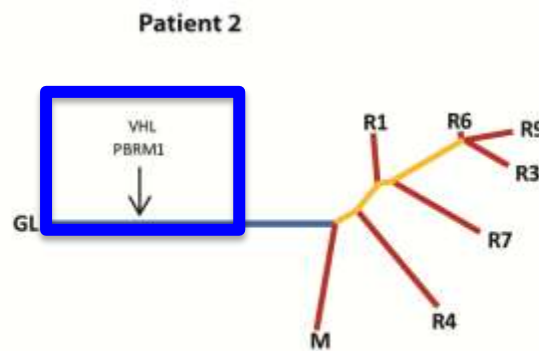
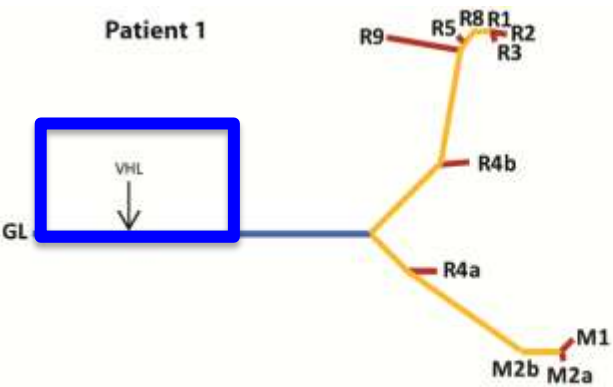




Mutations:

- Ubiquitous
- Shared
- Private

10  
Non-Synonymous Mutations



Mutations:

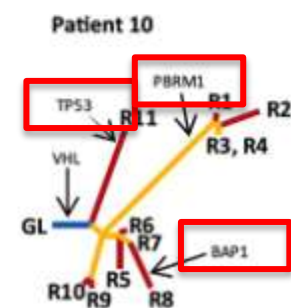
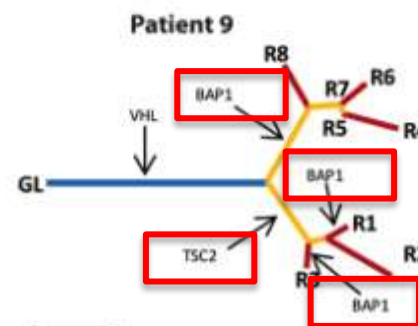
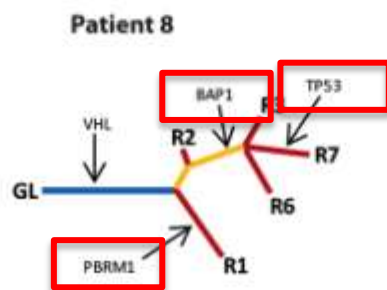
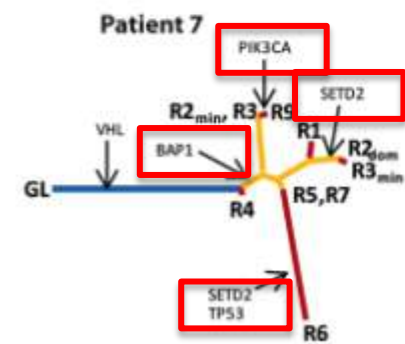
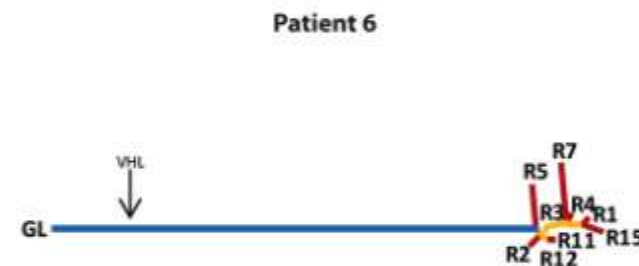
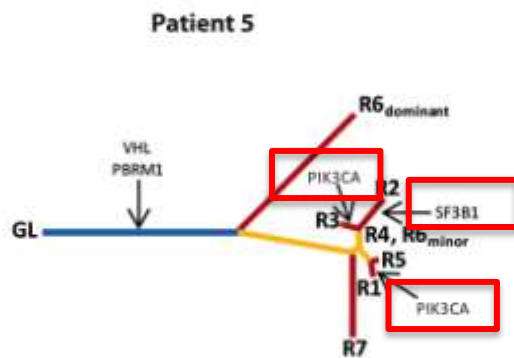
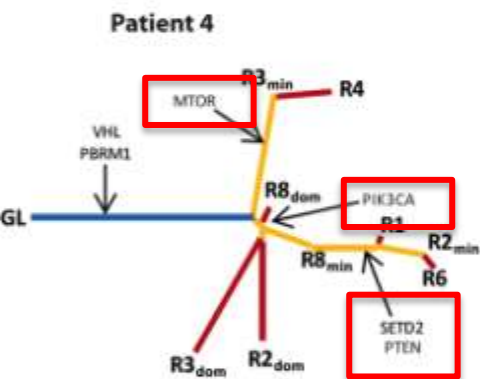
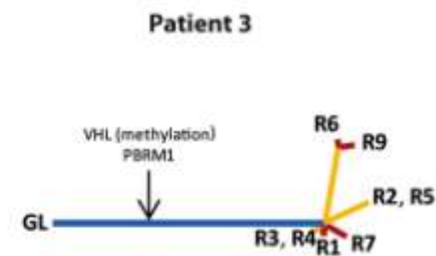
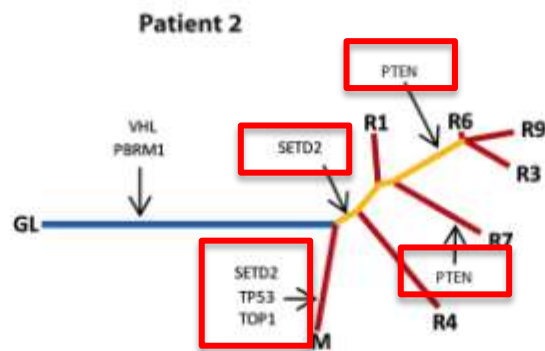
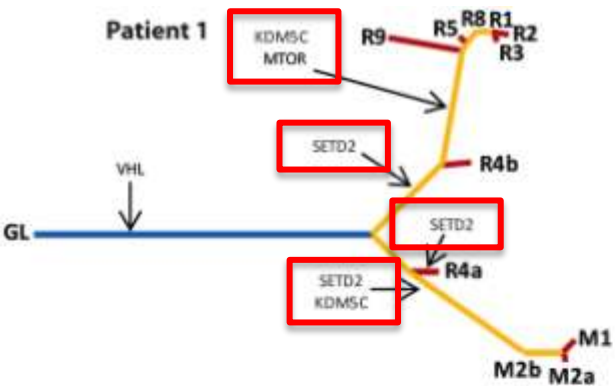
- Ubiquitous
- Shared
- Private

10  
Non-Synonymous  
Mutations

# Subclonal Driver Heterogeneity

## Confounding treatment success?





Mutations:  
 — Ubiquitous  
 — Shared  
 — Private

10  
 Non-Synonymous  
 Mutations

# Number of Driver Events under-estimated by Single Biopsy in ccRCC

## Driver Prevalence

	Per Biopsy TCGA n=164	Per Biopsy n=79	Per Patient n=10
<i>PBRM1</i>	42%	39%	60%
<i>SETD2</i>	18%	27%	30%
<i>BAP1</i>	21%	24%	40%
<i>KDM5C</i>	7%	11%	10%
<b>P53</b>	<b>5%</b>	<b>5%</b>	<b>40%</b>
<i>ATM</i>	3%	4%	10%
<i>ARID1A</i>	6%	1%	10%
<i>PTEN</i>	5%	10%	20%
<i>MTOR</i>	9%	8%	10%
<i>PIK3CA</i>	3%	4%	20%
<i>TSC2</i>	2%	4%	10%
PI3K/mTOR pathway	18%	28%	60%

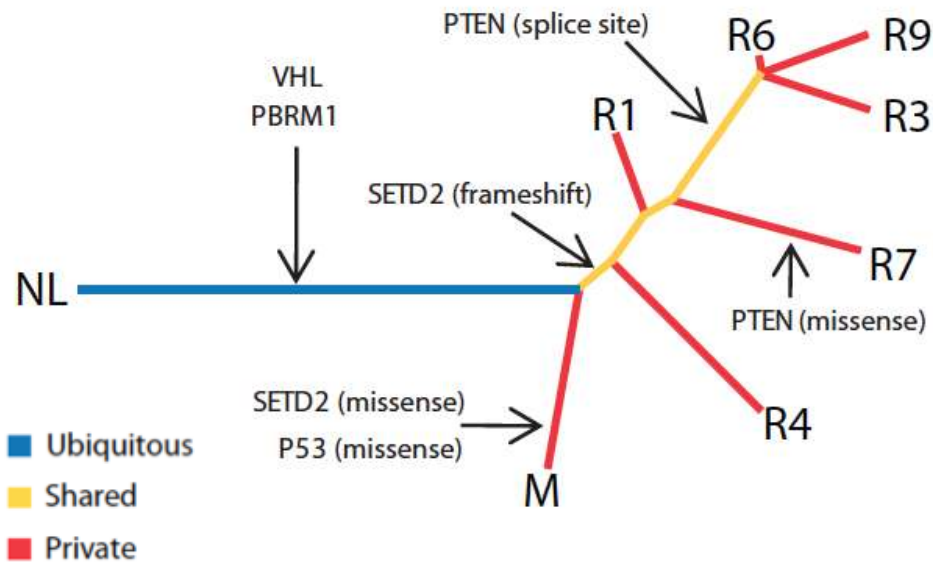
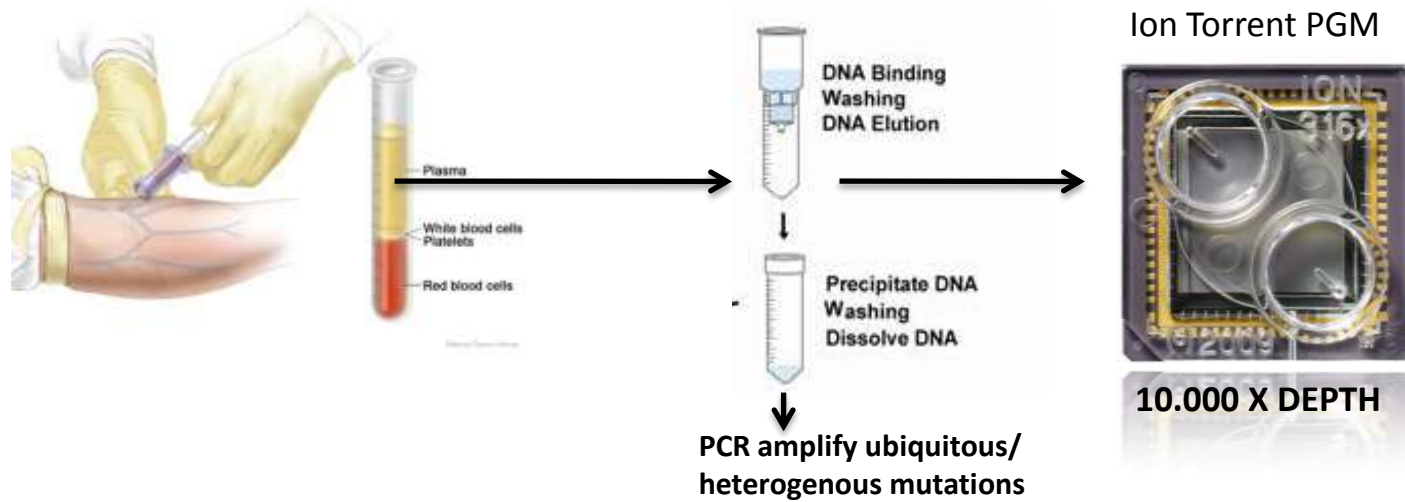
# How can the genomic landscape be more efficiently mapped?





# Tracking Tumour Subclonal Architecture Dynamics

## Somatic mutation detection in ctDNA extracted from peripheral blood.



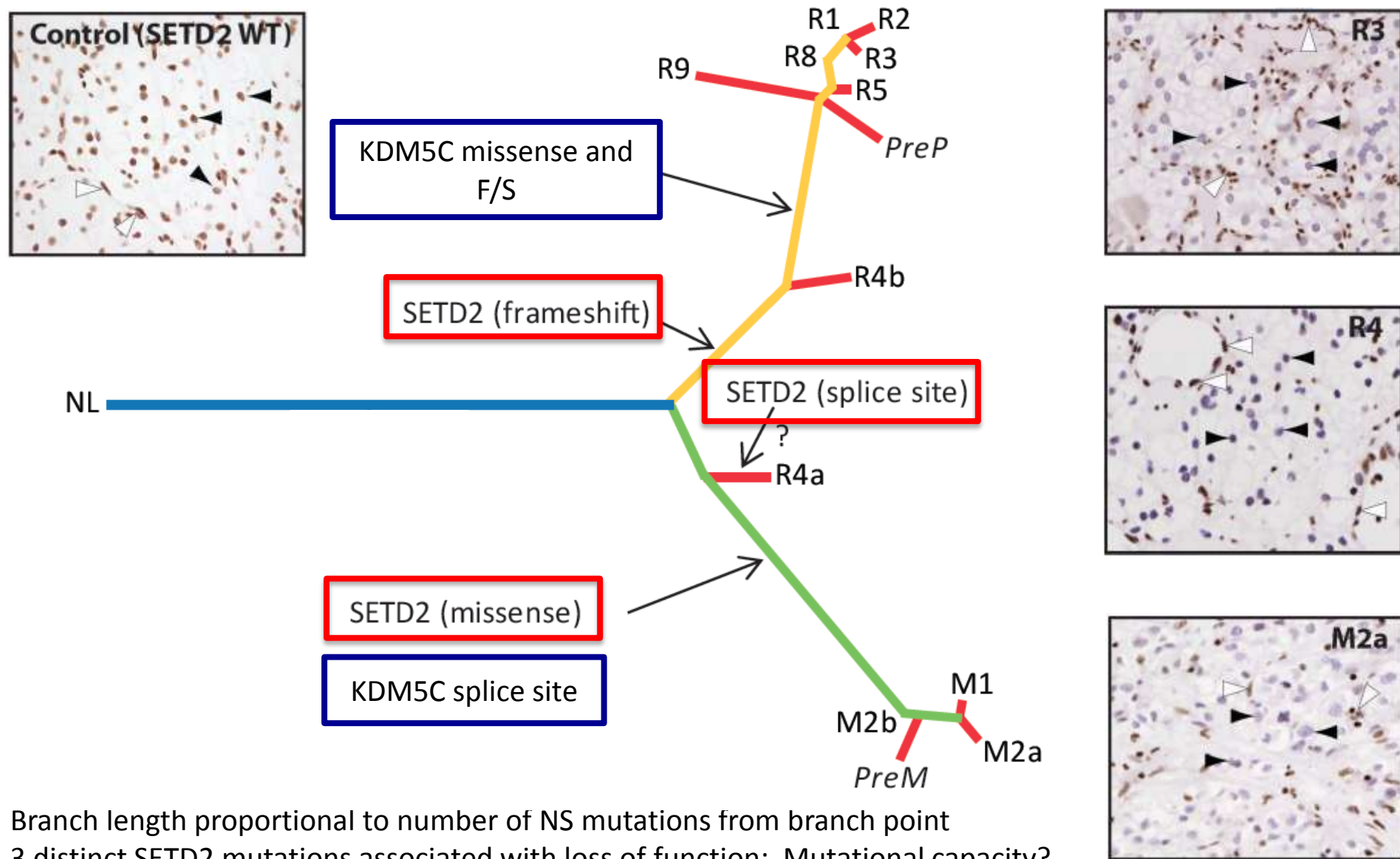
Regional Distribution	Gene	Variant Frequency
Ubiquitous	VHL	1.2%
R3, R6, R9	PTEN (splice)	3.7%
R3, R6, R9	ARHGEF7	0.9%
R3	SYNM	0.1%
R7	MGA	0.1%
R4	OR10H3	0.1%
M	SETD2	6.3%
M	TP53	0.2%

# Exploit Convergence of Driver Events

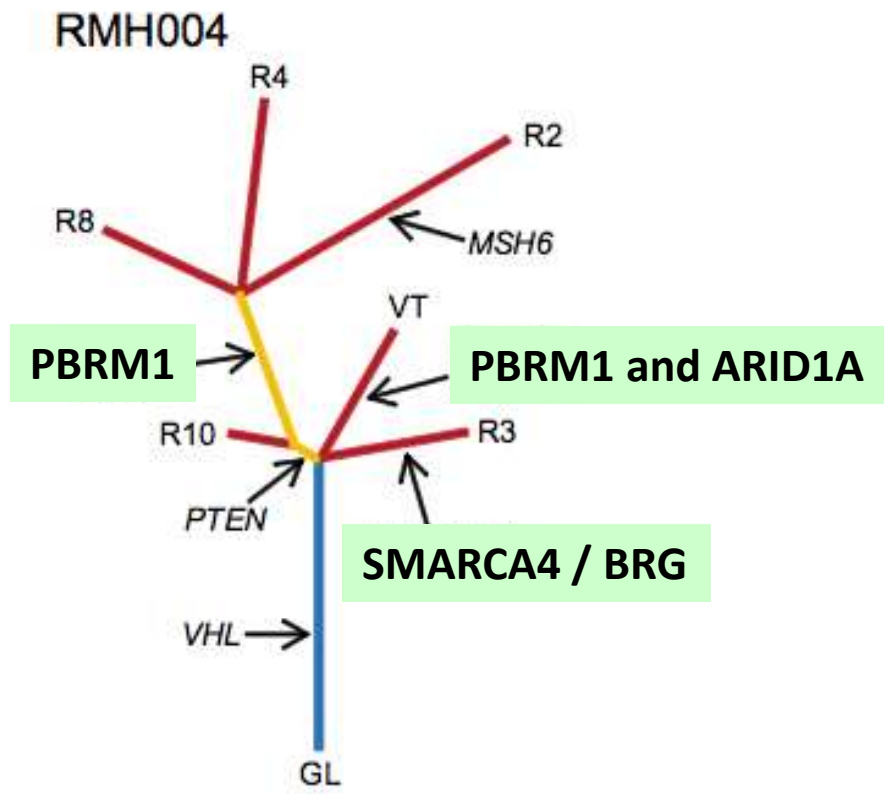


# Evidence for Parallel Evolution

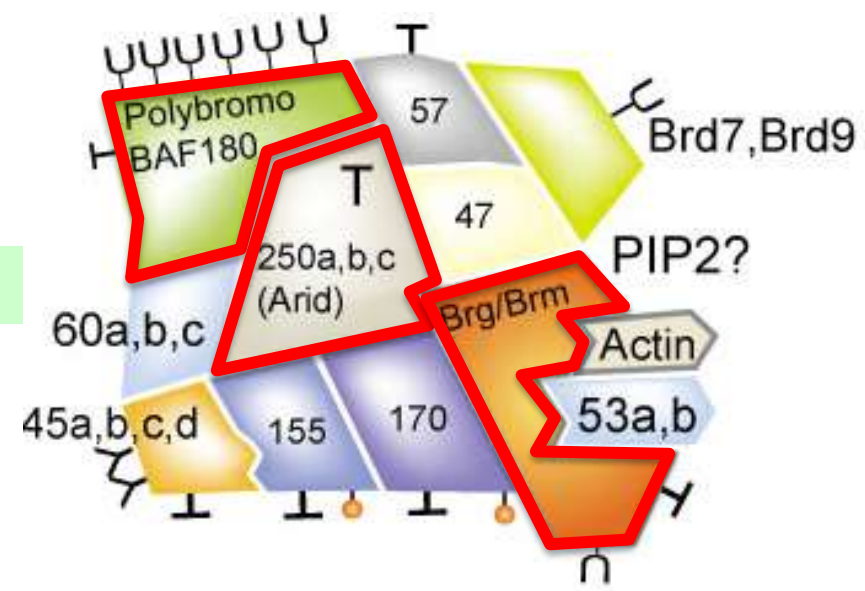
## SETD2 Loss of Function: H3K36 tri-methylation



# Genetic Heterogeneity may affect the same **Protein Complex** in different branches



## SWI/SNF Chromatin remodeling Complex



# Disruption of the mTOR Pathway

## A Common Convergent Event



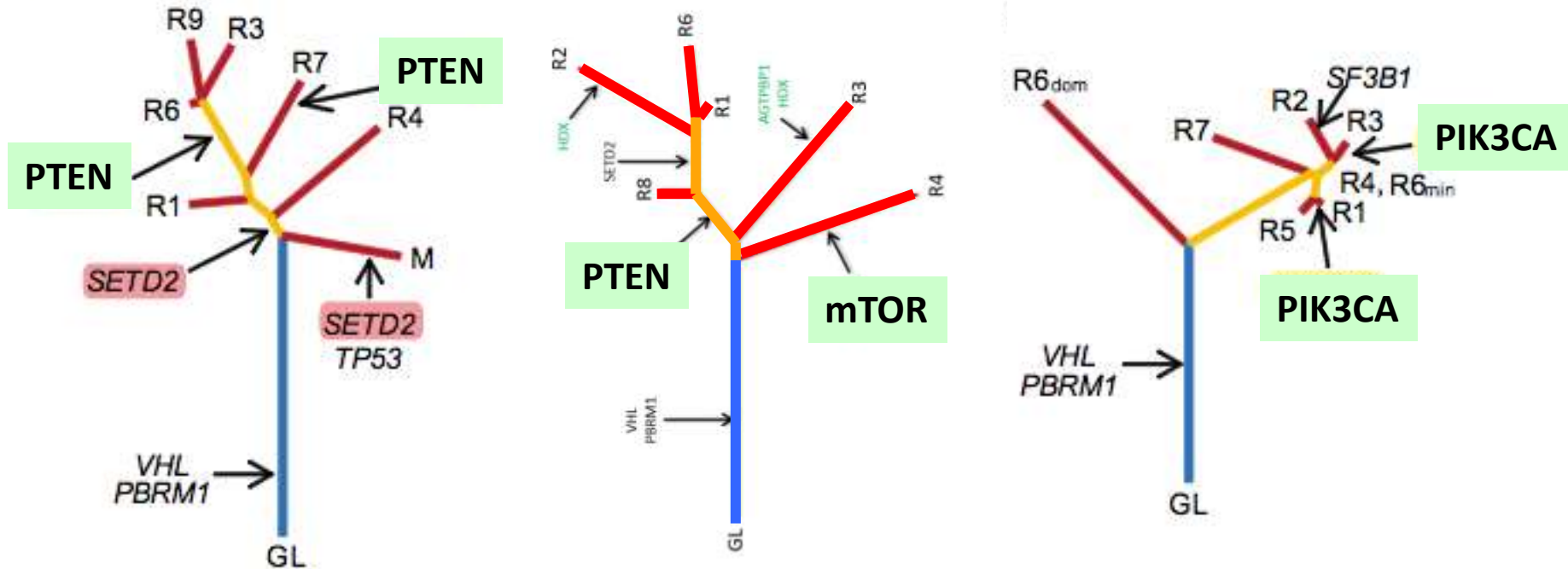
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PI3K/mTOR pathway } 18%		} 28%	
} 60%		} 60%	

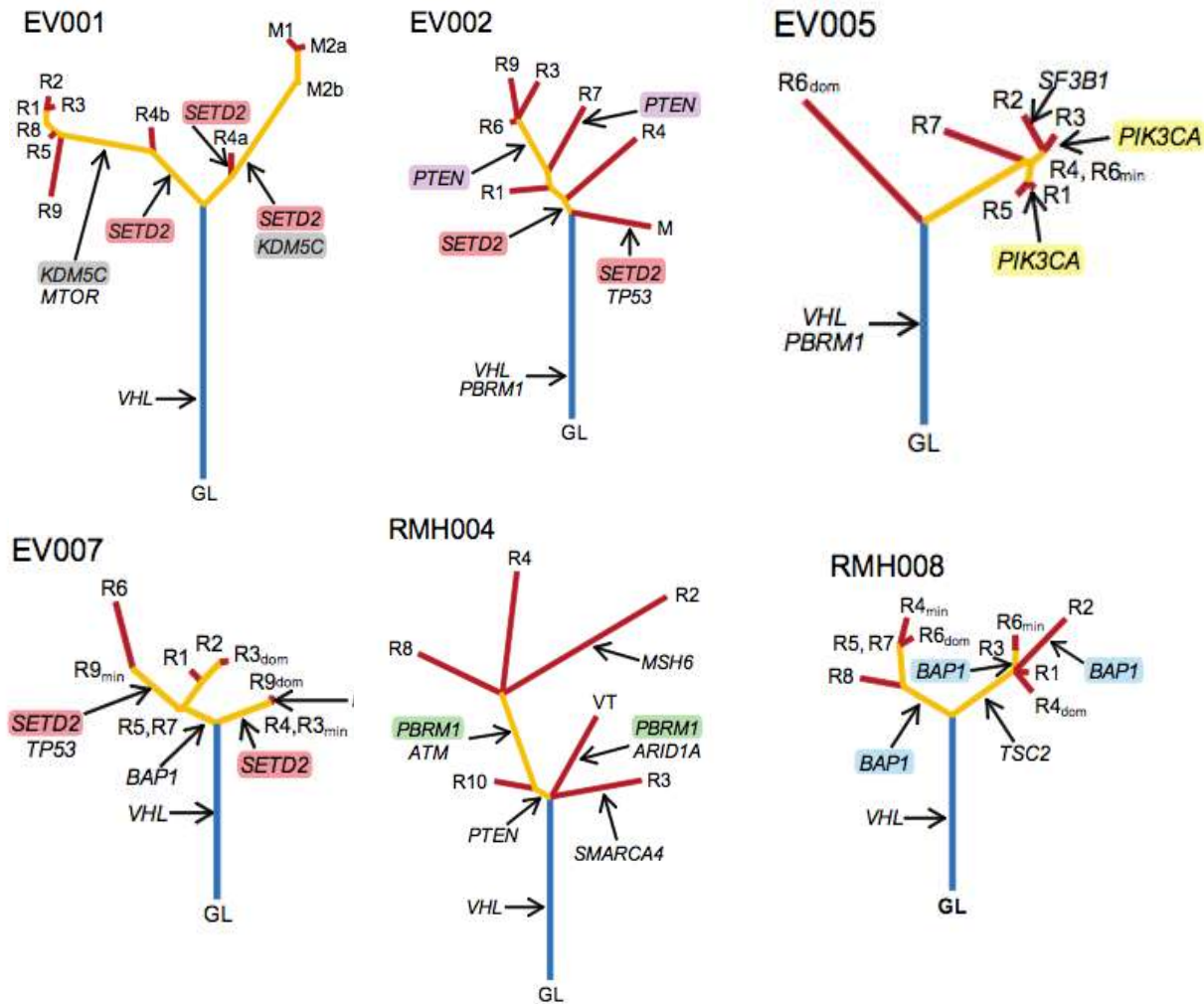
mTOR pathway

# Genetic Heterogeneity may affect the same **Signaling Pathway** in different branches



- Genetic heterogeneity may impact the same signalling pathway in different branches
  - PI3K/PTEN/mTOR

# Genetic Heterogeneity commonly affects the same **Driver** in different branches

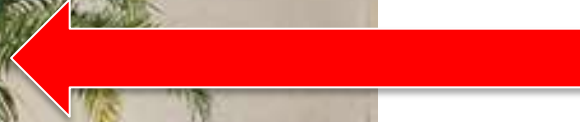


- 6/10 Tumours have recurrent mutations in the branches in the same gene
  - PIK3CA, PTEN,
  - SETD2, KDM5C, BAP1, PBRM1



# Target Tumour Phylogenetic Trunks and Resolve Branches

Target convergent events in the branches

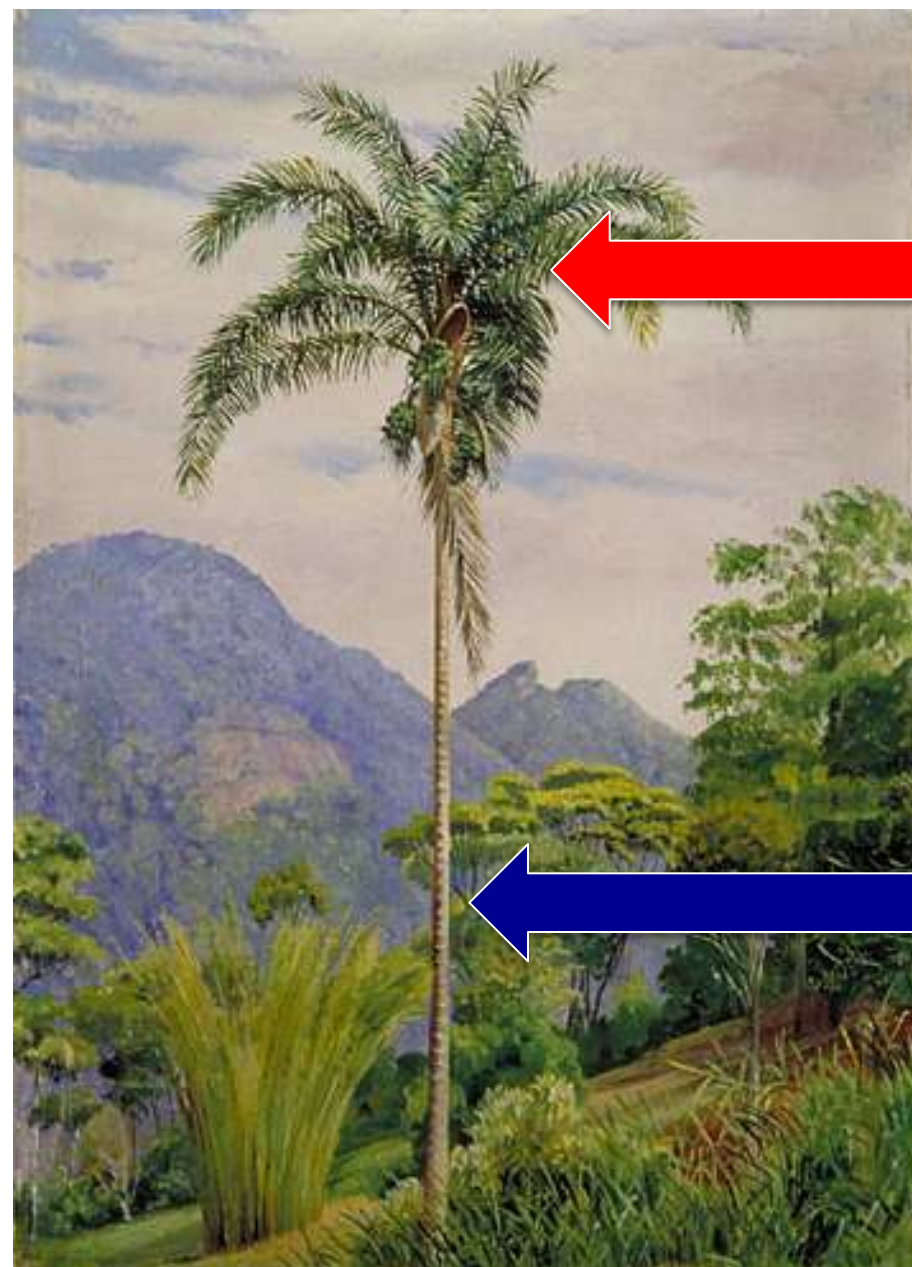


Target lethal subclone(s)

Trunk Genetic Events Present in  
Every Cancer Cell



**DEFINE and TARGET TRUNK DRIVERS**

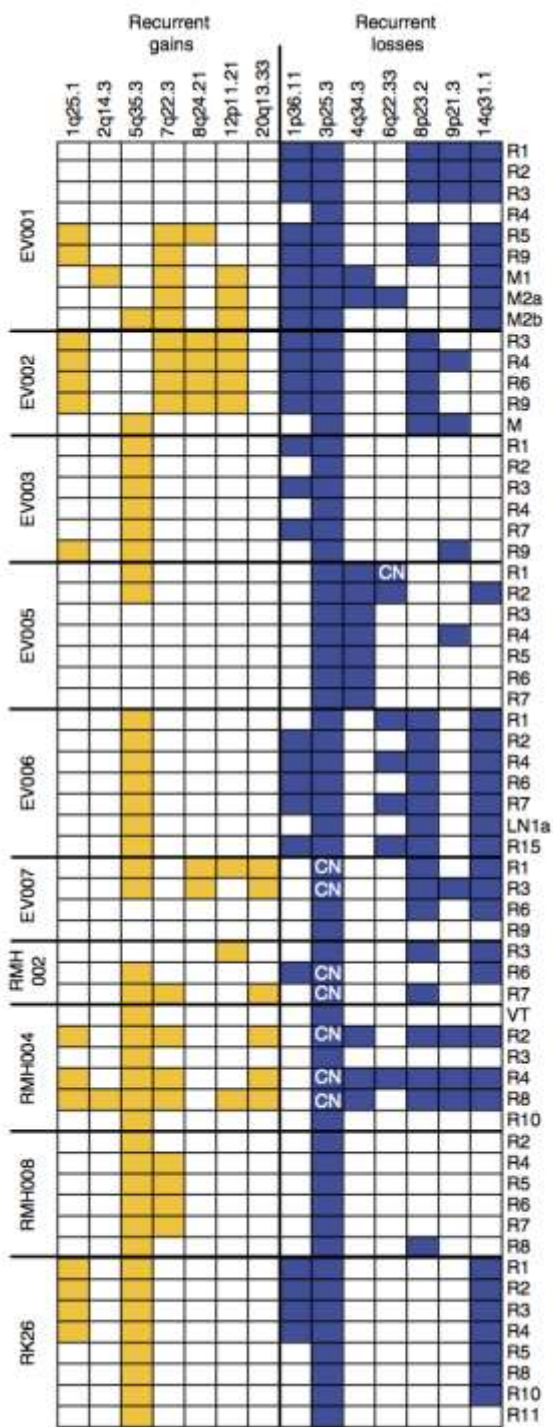


# Subclonal SCNA Heterogeneity

## Confounding treatment success?

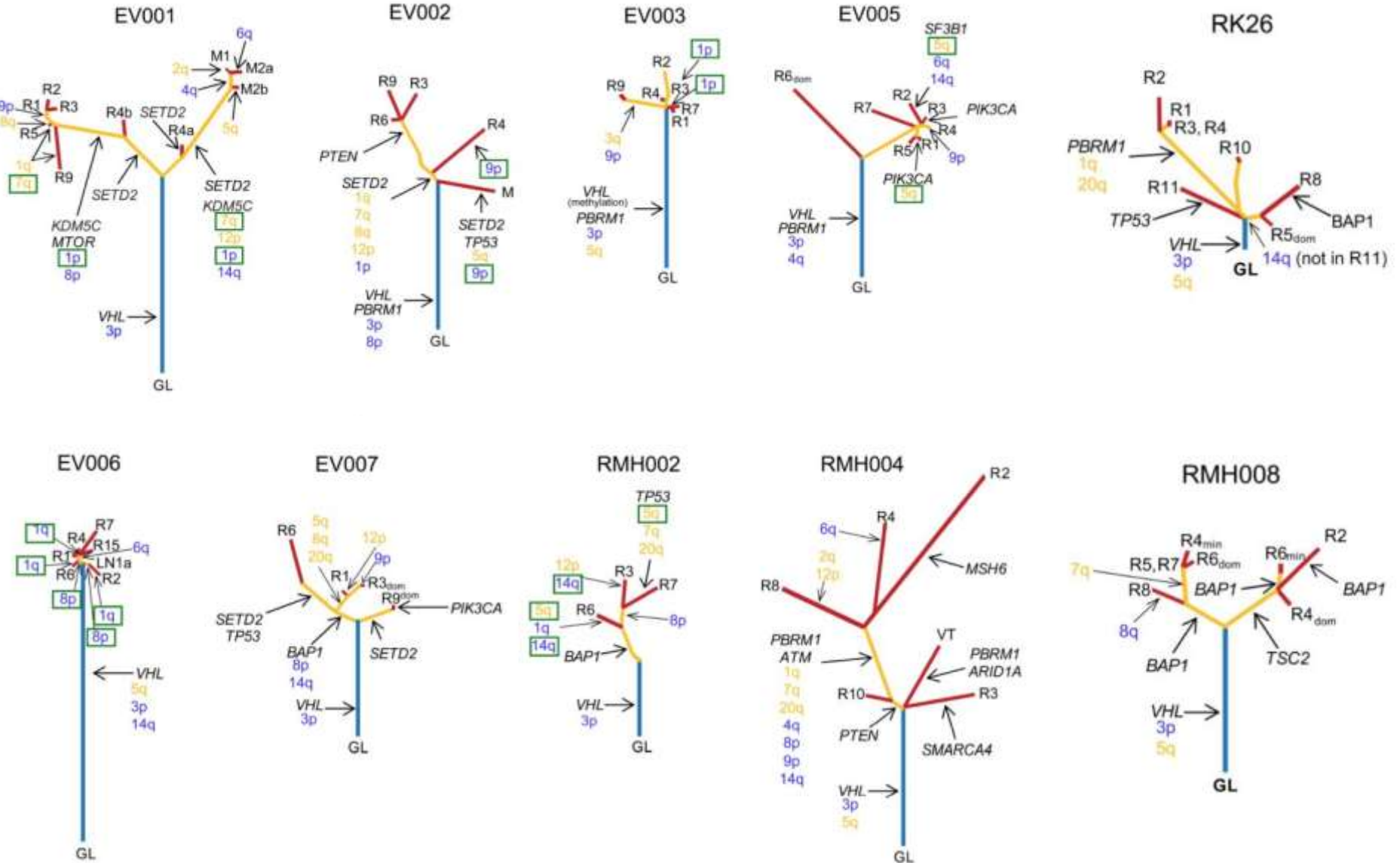


# Trunk and Branched SCNAs



Case	Driver CNVs	Heterogeneous driver CNVs (%)
EV001	13	12 (92.3)
EV002	9	7 (77.8)
EV003	5	3 (60.0)
EV005	6	4 (66.7)
EV006	6	2 (33.3)
EV007	8	7 (87.5)
RMH002	8	7 (87.5)
RMH004	12	10 (83.3)
RMH008	4	2 (50.0)
RK26	5	3 (60.0)
Total	76	57 (75)

# SCNAs Mapped to Phylogenetic Trees



# Tumour Sampling Bias

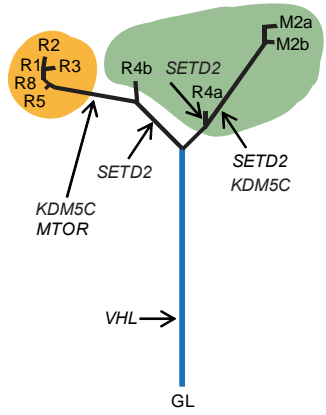
Difficulties of identifying uniform biomarkers



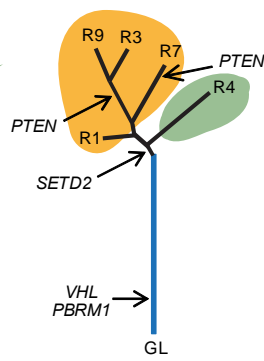
# Heterogeneous Nature

CCB Poor Prognostic Signature  
and  
CCA Good Prognostic Signature

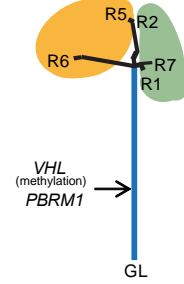
EV001



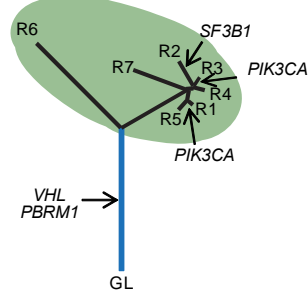
EV002



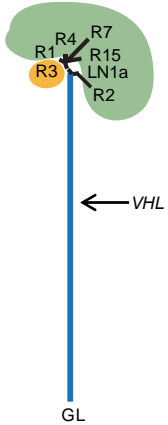
EV003



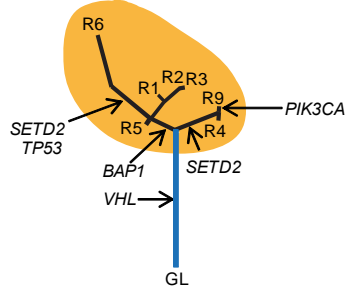
EV005



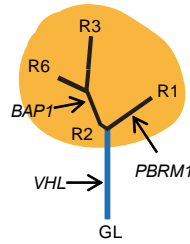
EV006



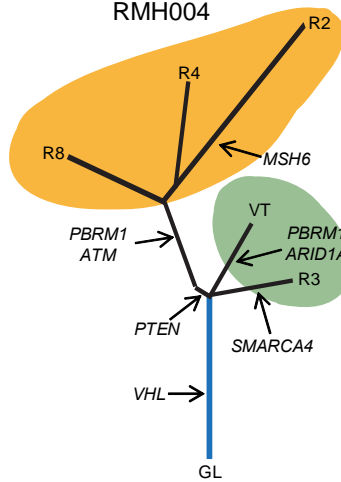
EV007



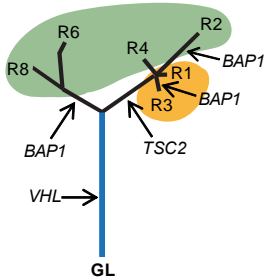
RMH002



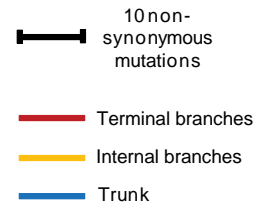
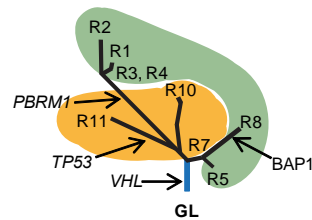
RMH004



RMH008



RK26



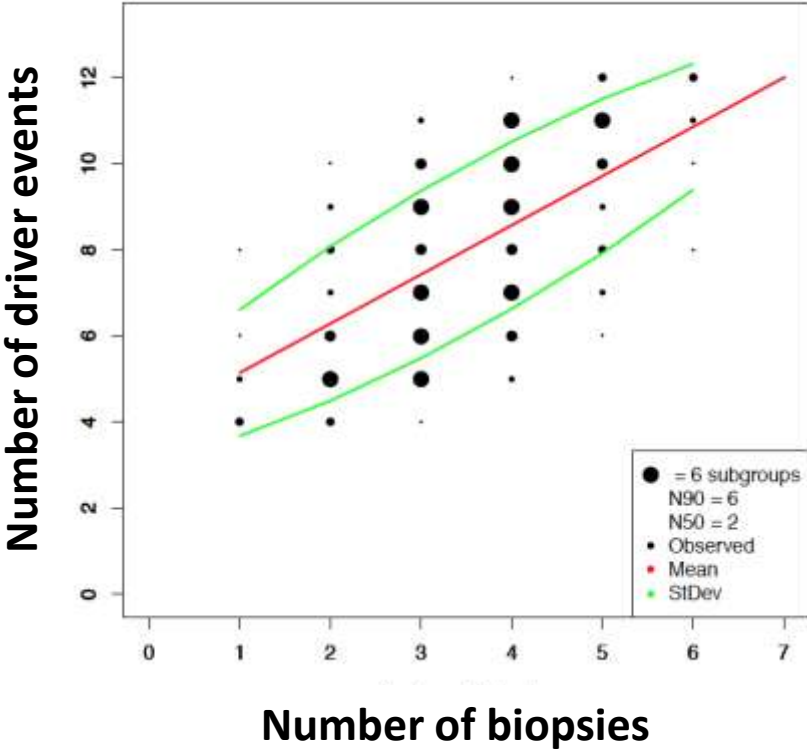
# Mapping the Genomic Landscape of ccRCC

## How deep is the rabbit hole?

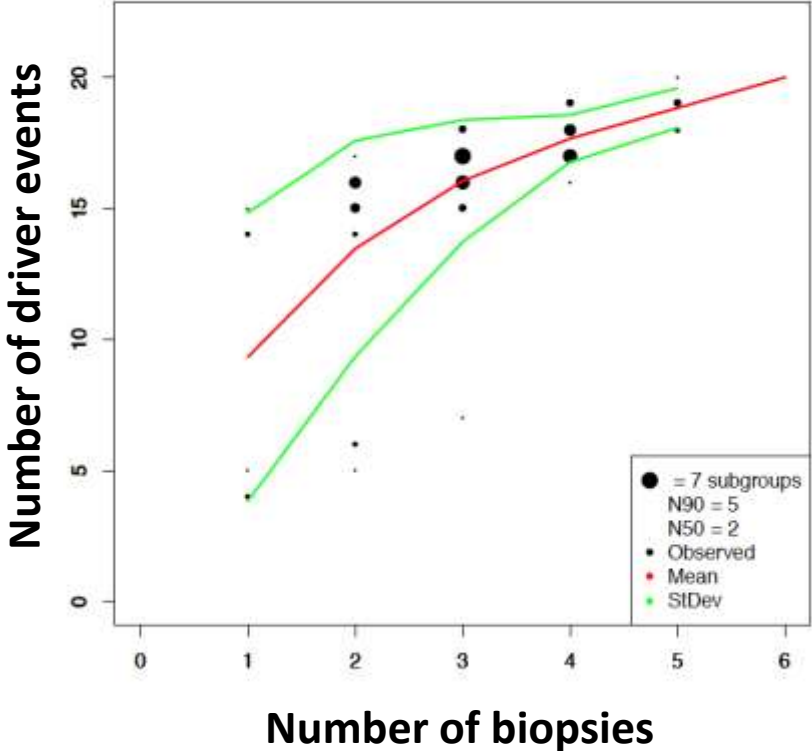


# How many biopsies to fully map the number of driver events?

EV005 Driver variants



RMH004 Driver variants





# Clinical Implications of Driver Heterogeneity

- >70% of Drivers are heterogeneous and spatially separated (BAP1, SETD2, PBRM1, KDM5C, MTOR, PIK3CA, PTEN, TSC2, TP53)
- Subclonal drivers cannot be readily distinguished in single biopsies
- Current sampling techniques are underestimating number of driver events in RCC
- Similar trunk driver events but diverse patient outcomes:
  - Branches and subclonal drivers are likely to influence outcome



# TRACERx

TRacking Cancer Evolution through Therapy (Rx)

# Functional and Genetic Intratumour Heterogeneity

- Optimise Clinical trial development: Target Trunk vs Branched drivers
- Exploit constraints to cancer evolution
  - parallel evolutionary events converging on single genes or pathways
- Define relationships between diversity, clinical stage and cancer outcome
- Autopsy Programs: Define the origins of the lethal subclone
- Impact of therapy on cancer genome evolution
- Develop non-invasive methods to monitor cancer evolution: cfDNA
- Identify sequence of somatic events in relation to genome instability onset:
  - Define drivers of diversity and genome instability in tumours

# Renal Cancer Evolution Summary

- Need to address two principles of Darwinian evolution
  - Decipher mechanisms of cancer **diversity**
  - Improve cancer **selection** pressures:
    - **Target Clonal Driver VHL loss of function,**
    - **Resolve subclonal dynamics**
- Understand evolutionary pressures resulting in parallel evolution of subclones
  - Develop better animal models of ccRCC
- Longitudinal cancer cohort studies (TRACERx): reveal processes shaping tumour genome evolution
  - **Define Origins of lethal subclone**

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