USTAR Center for Genetic Discovery

Tracking cancer evolution across time and space

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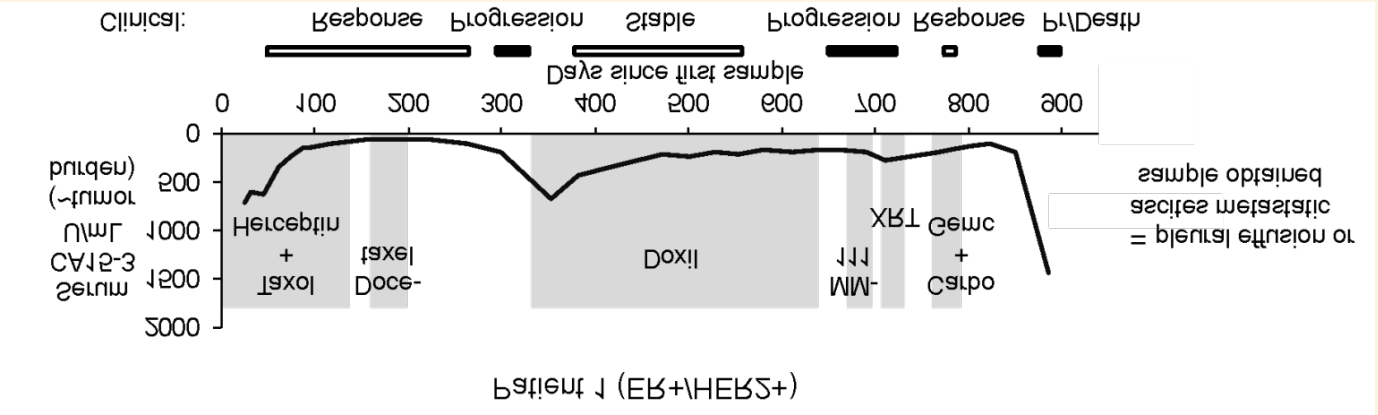
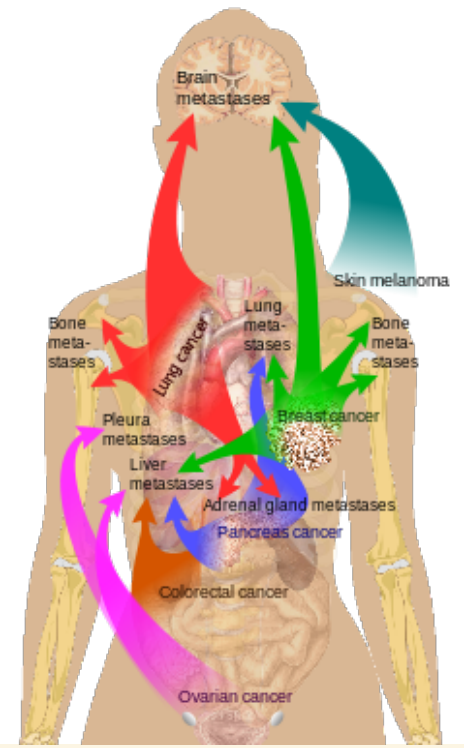
We want to understand how a patient's tumor evolves…

… during disease progression and relapse at a single tumor site, across multiple courses of chemotherapy





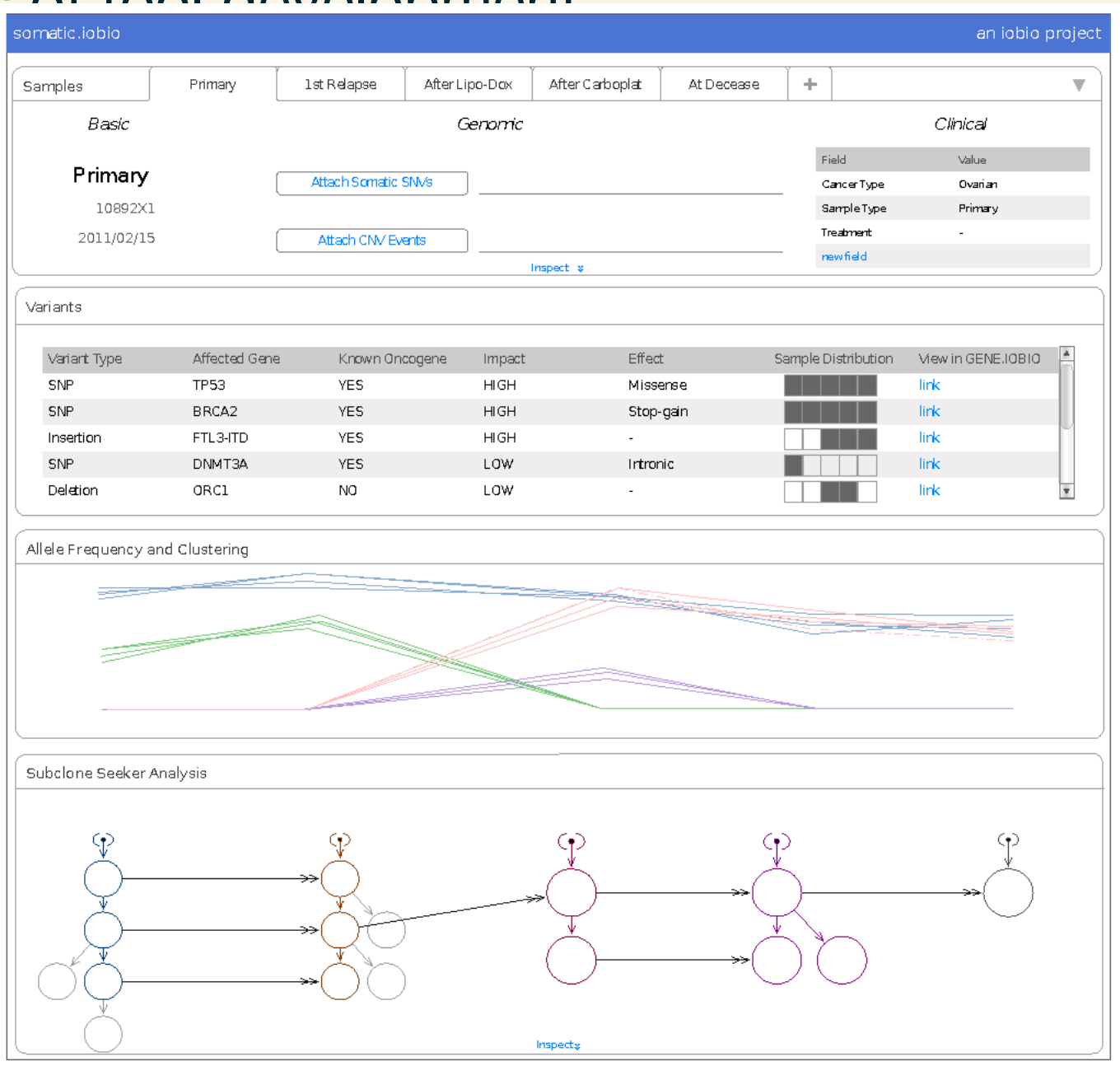
… and across metastasis, while colonizing distant organs and establishing multiple metastatic sites.

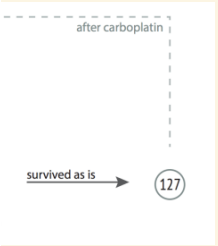
Cancer is a genetic disease

Four distinct areas of tool development

1. Detect all somatic mutations and inherited variants

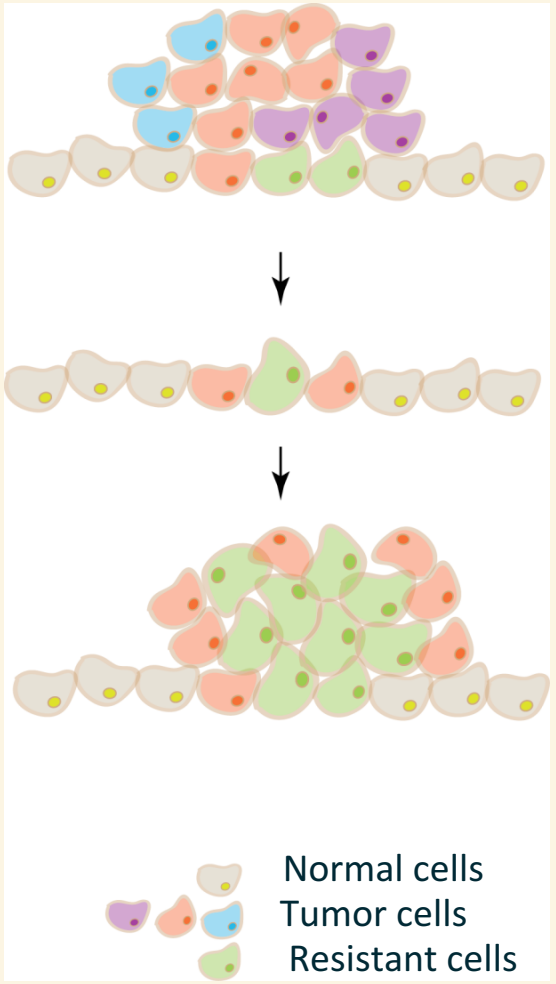
3. A mu int





The main steps of subclone analysis: a *primer*

Chemo-naïve primary tumor



Chemotherapy (taxol + carboplatin)

Resistance and regrowth

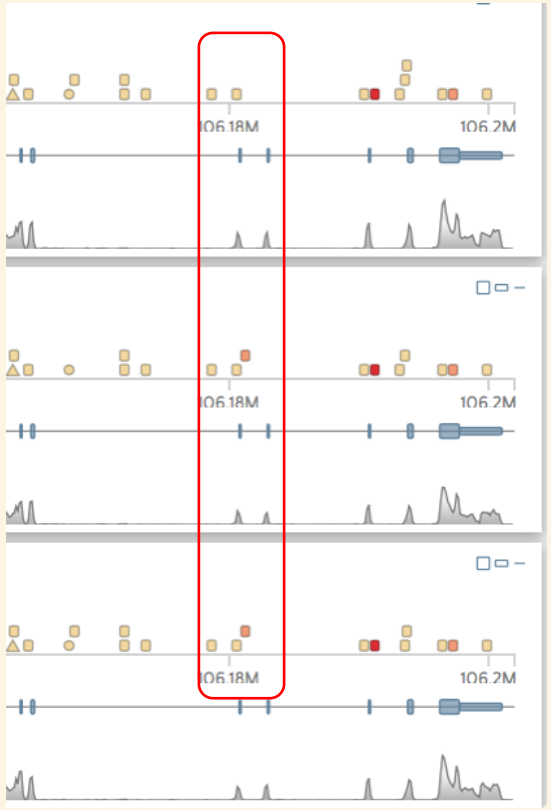
Chemo-resistant tumor

1. Detect somatic mutations

Normal tissue (blood)

Primary tumor

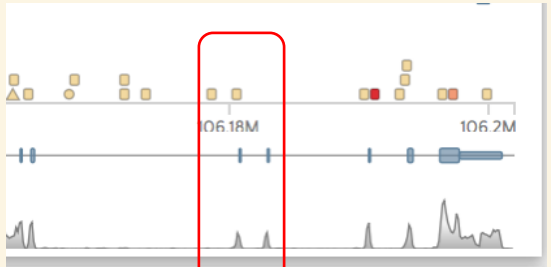
Relapse tumor



1. Detect somatic mutations

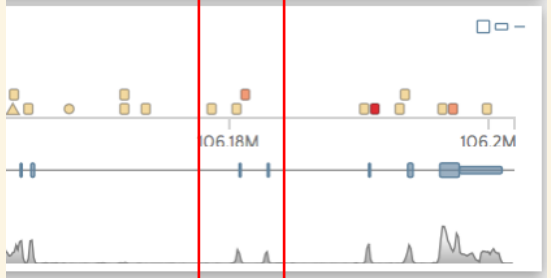
2. Determine mutation allele frequency (AF)

Normal tissue (blood)



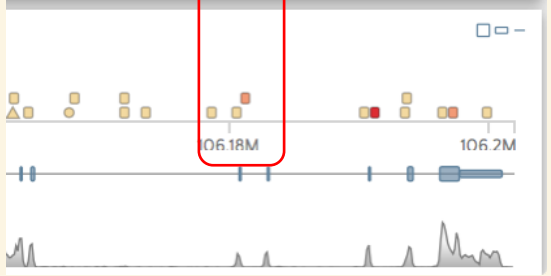
0%

Primary tumor



30%

Relapse tumor



40%

1. Detect somatic mutations

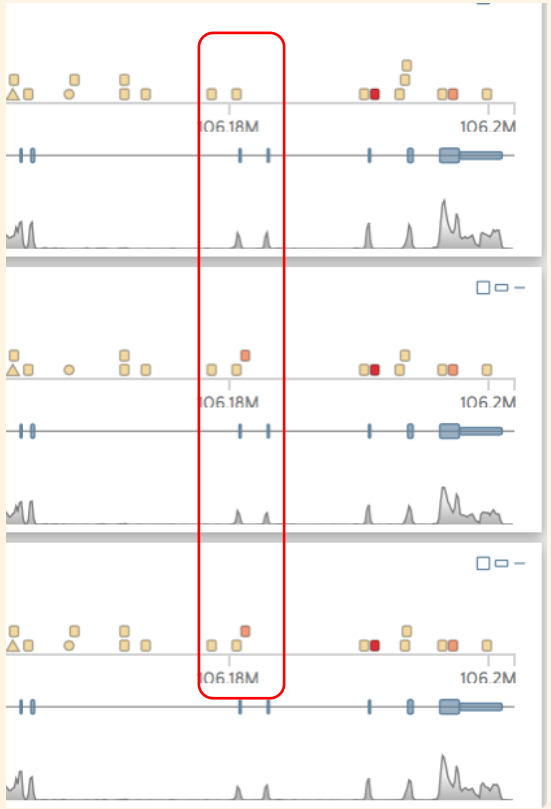
2. Determine allele frequency (AF)

3. Convert to cellular prevalence (CP)

Normal tissue (blood)

Primary tumor

Relapse tumor



0%

30%

40%

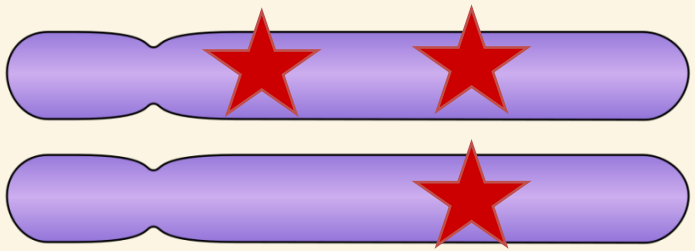
0%

60%

80%

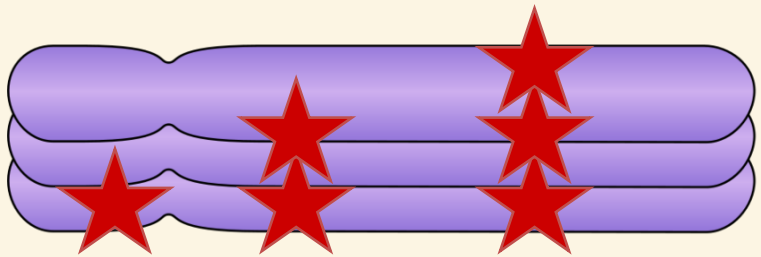
Example: AF=10%

Normal copy number (CN=2)



20% 10%

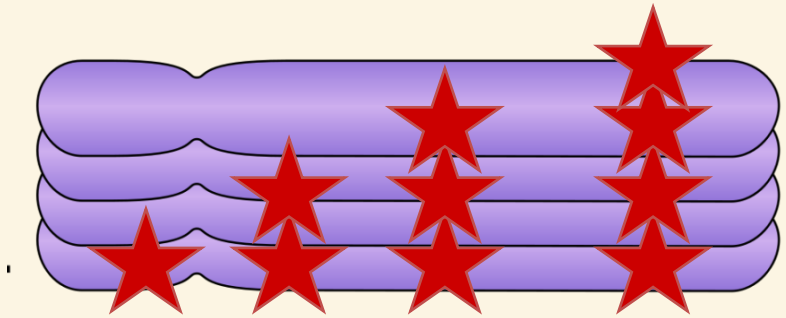
Amplification (CN > 2)



30% 15% 10%

Deletion (CN < 2)

10%

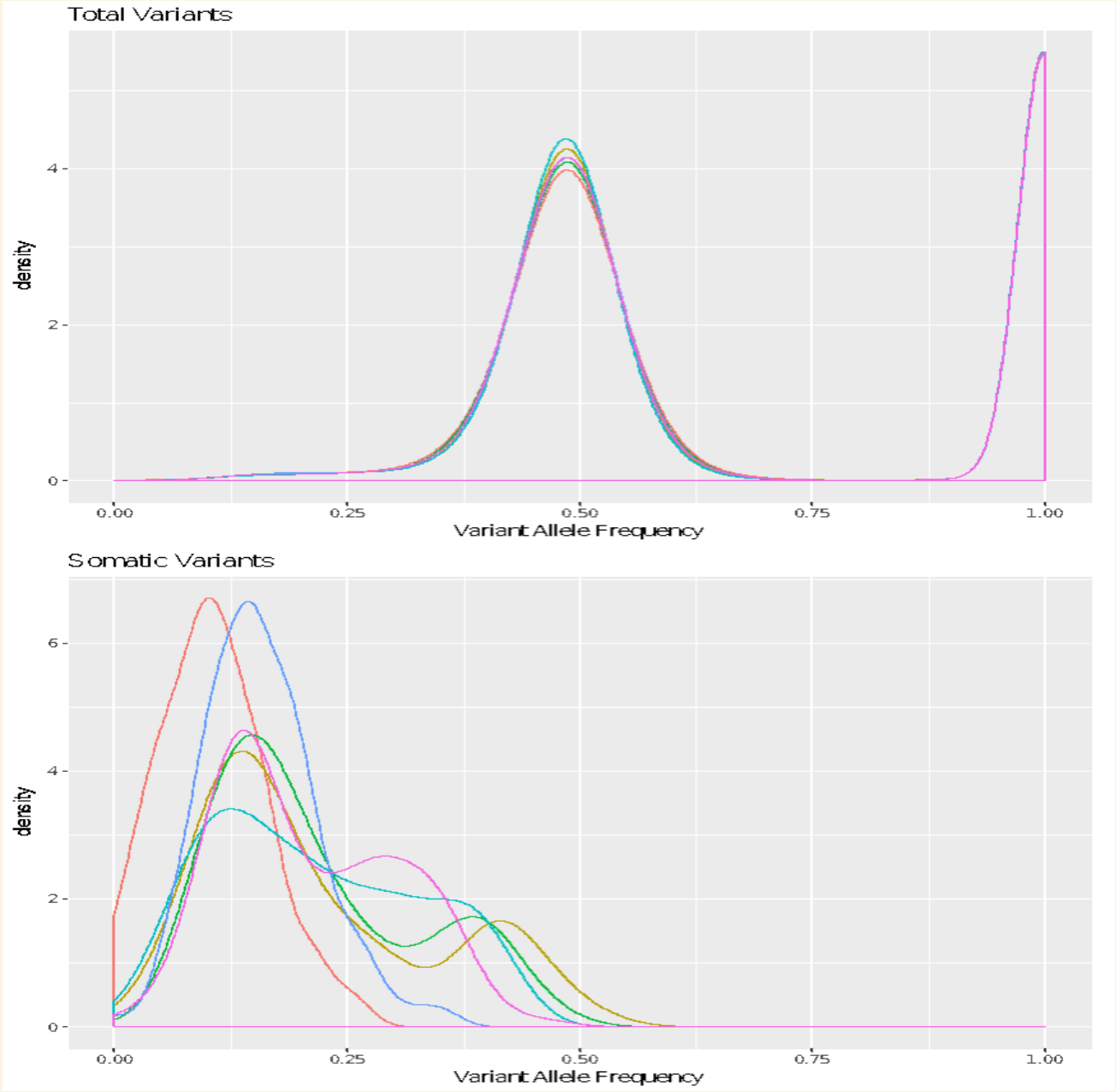


40% 20% 13.3% 10%

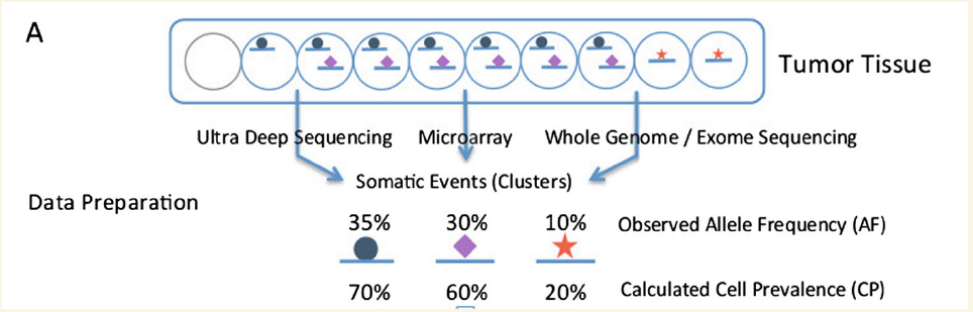
3. Mutation clustering

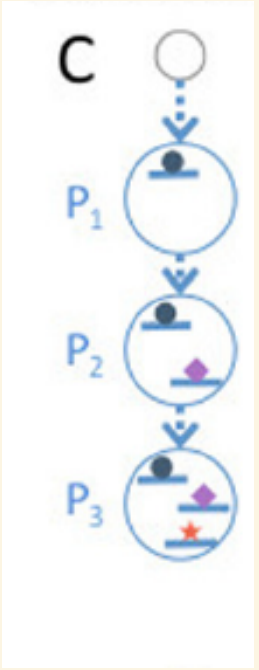
Inherited variants

Somatic mutations



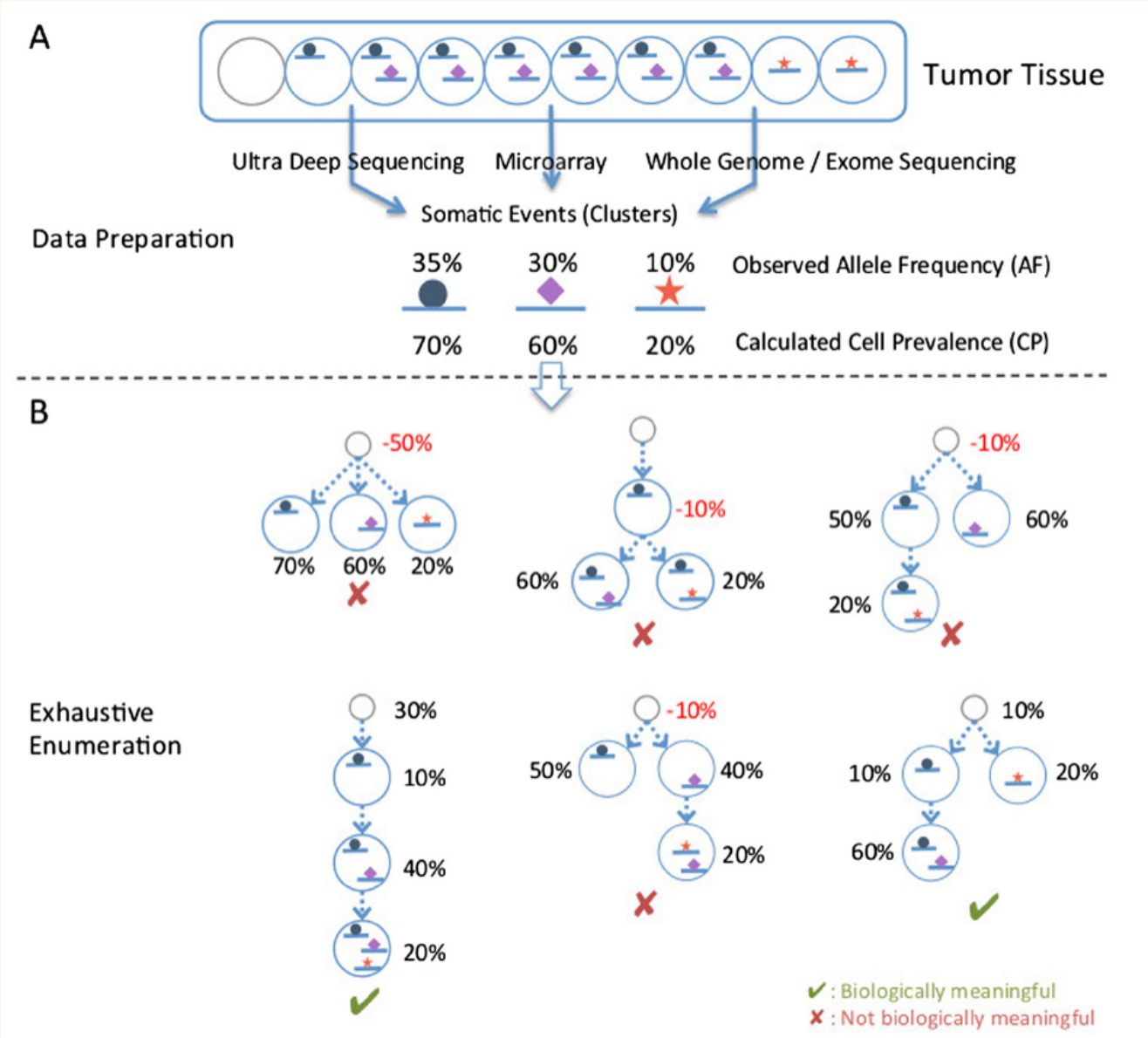
5. Subclone inference (reconstruction)



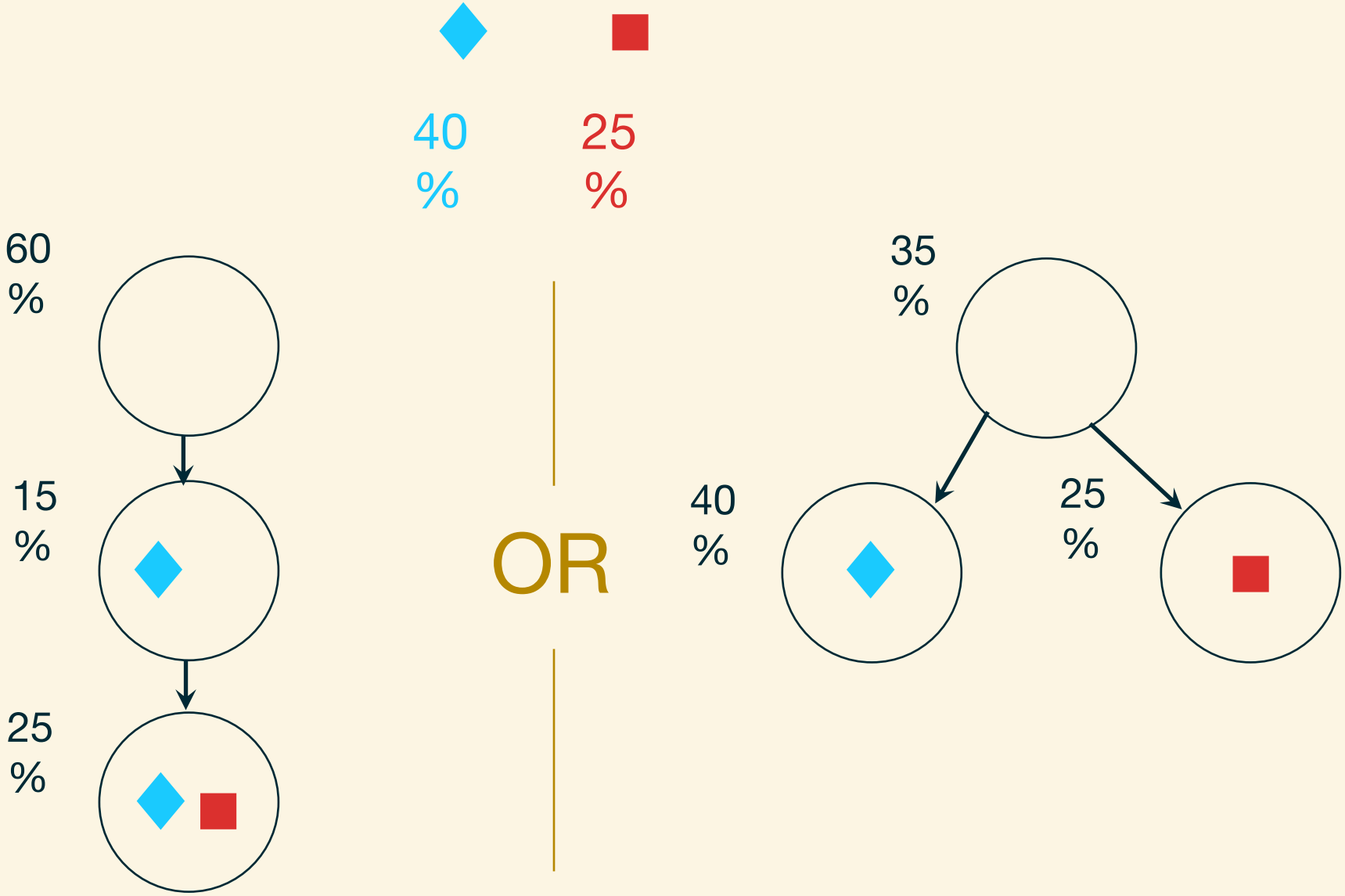


Our SubcloneSeeker method identifies all subclone evolutionary "trajectories" consistent with the bulk mutation AF data

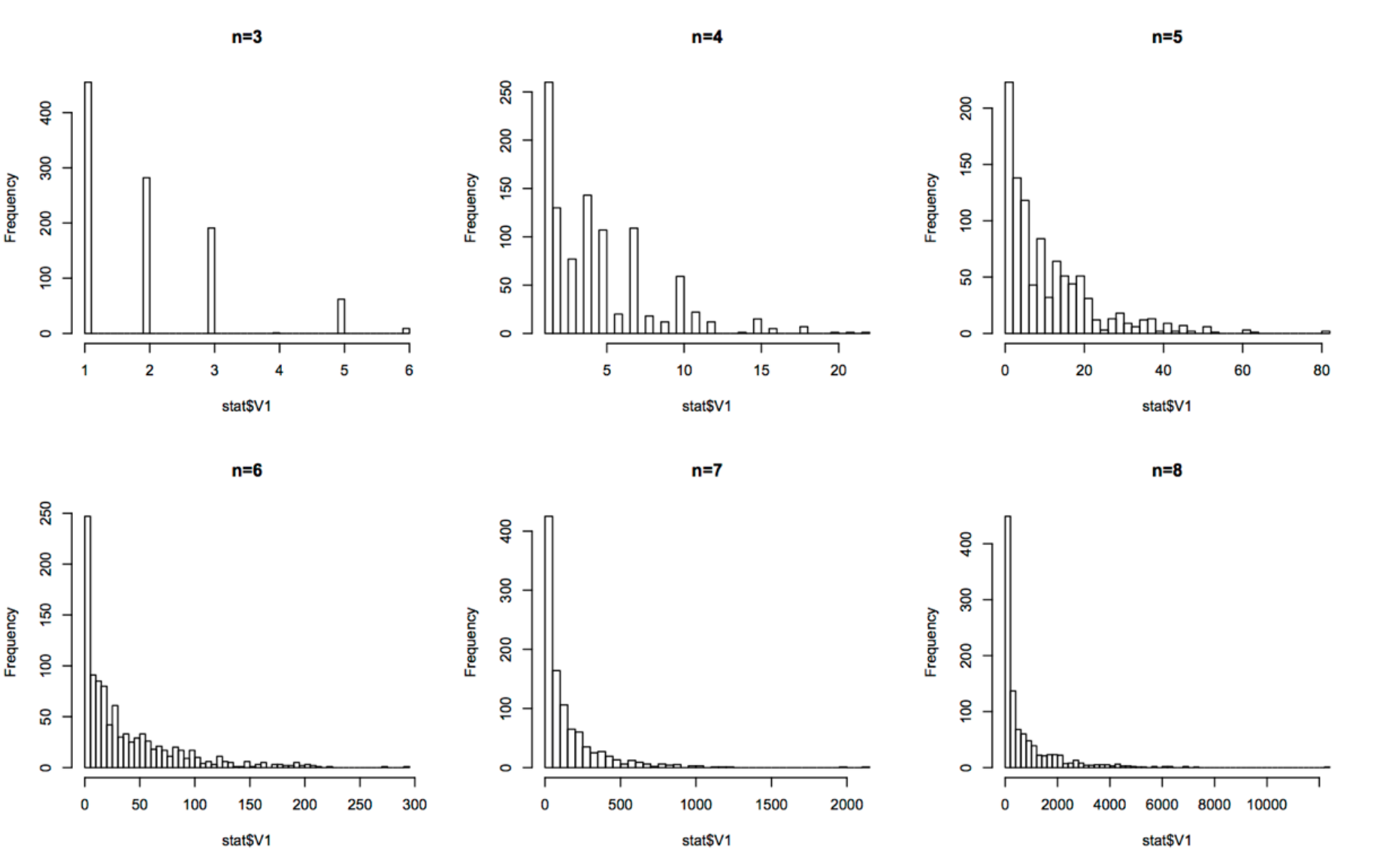
Qiao et al., **SubcloneSeeker**, Genome Biology, 2014



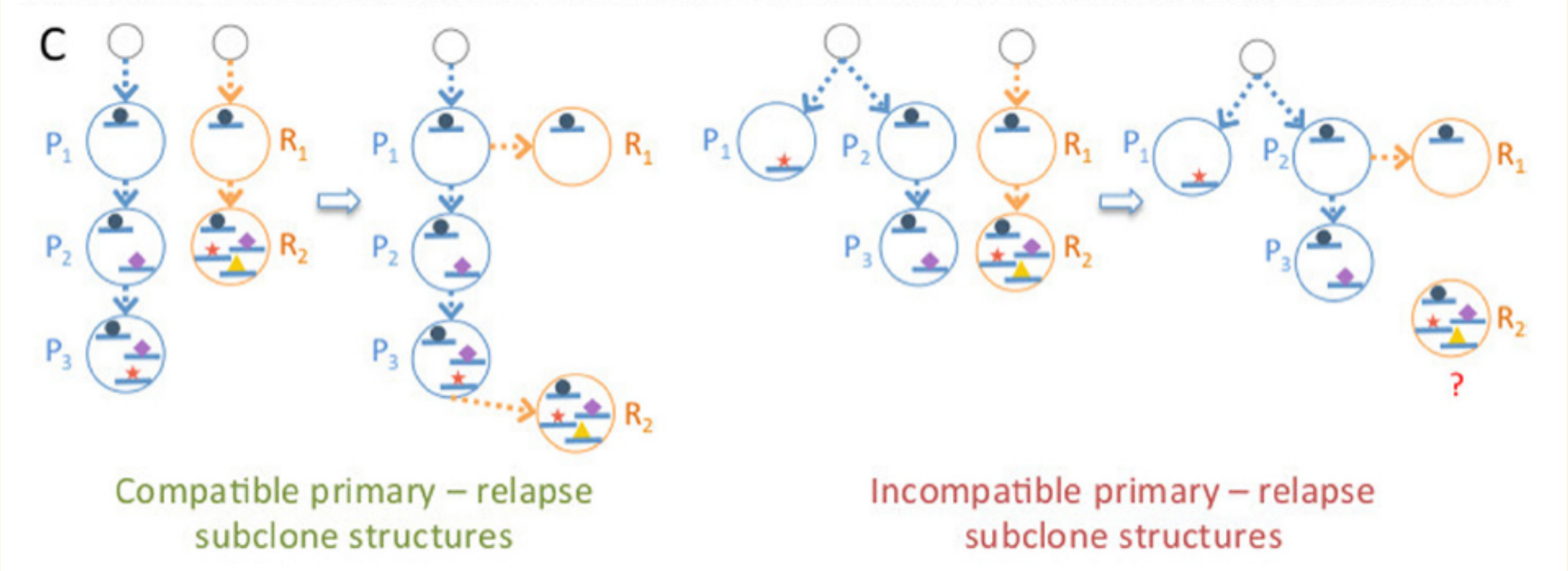
Multiple subclone structures may account for bulk data



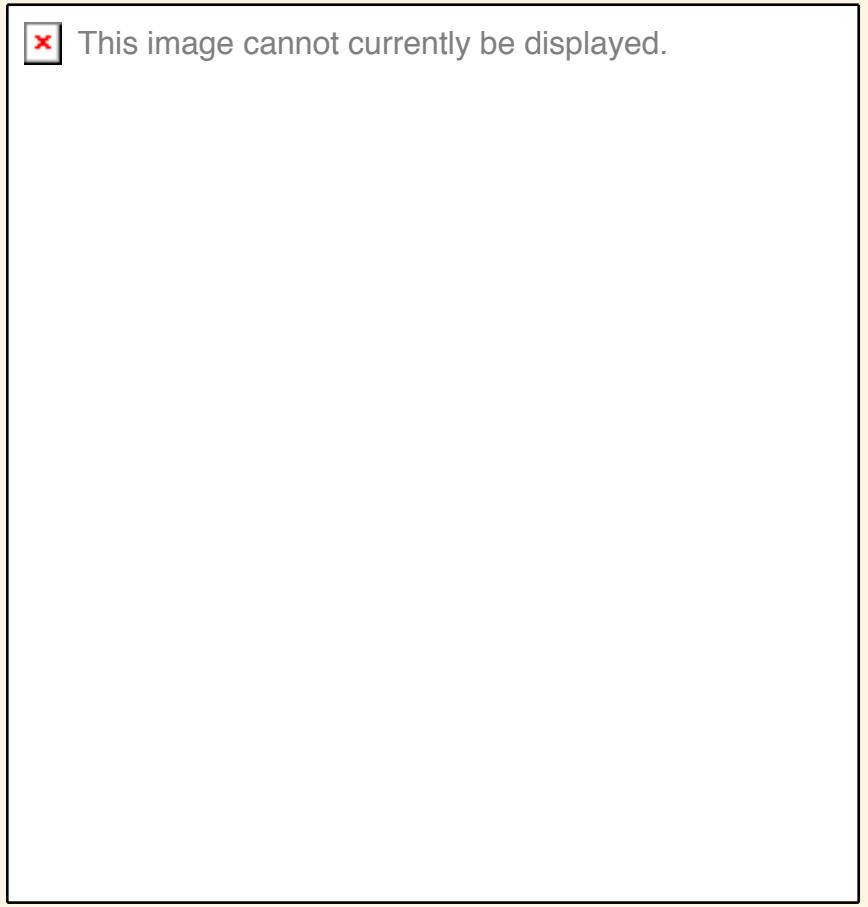
Number of “consistent” subclone structures is small

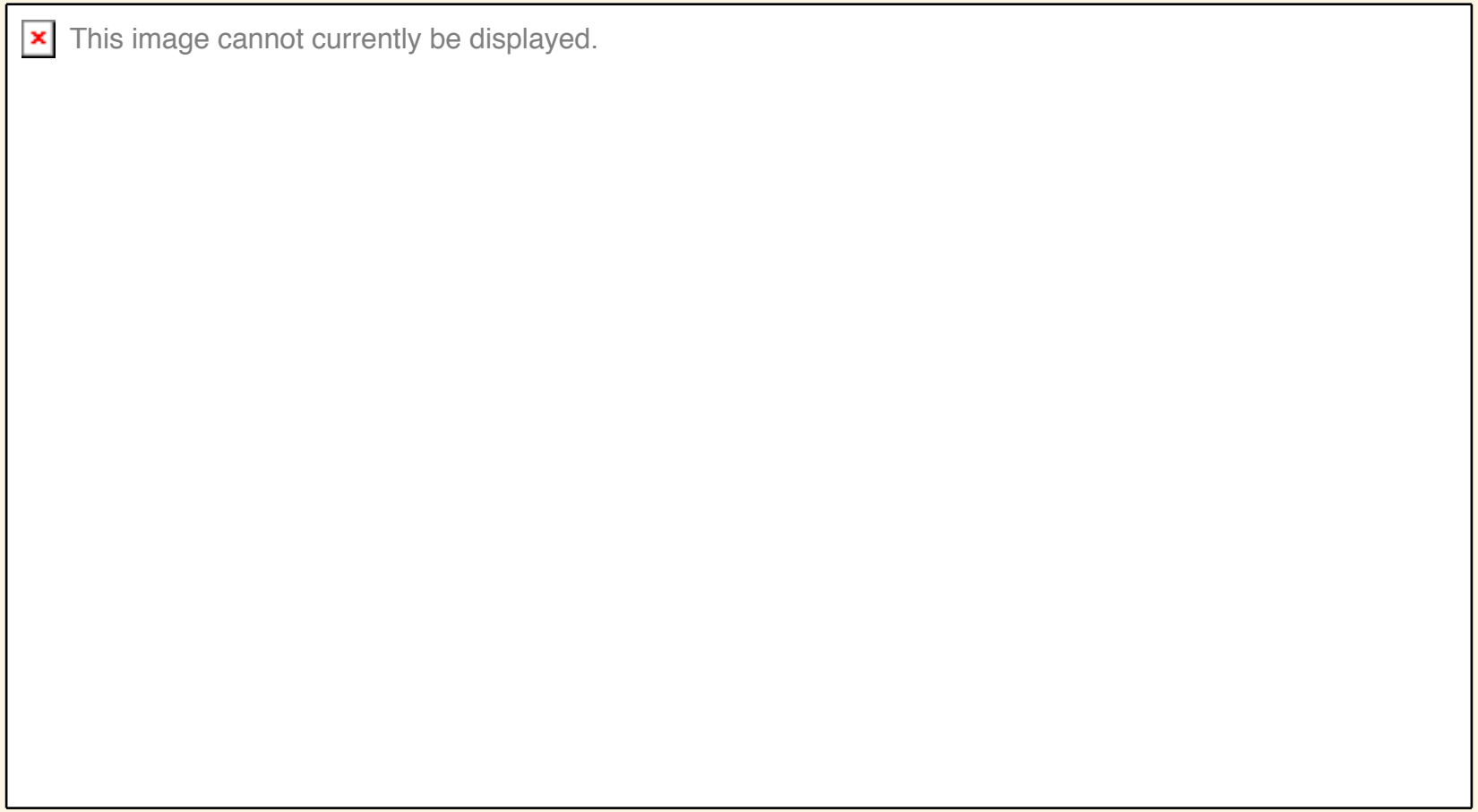


Further reduction of the “solution space” is possible with multiple biopsies from the same patient



Back to our primary chemonaïve / resistant relapse ovarian example:

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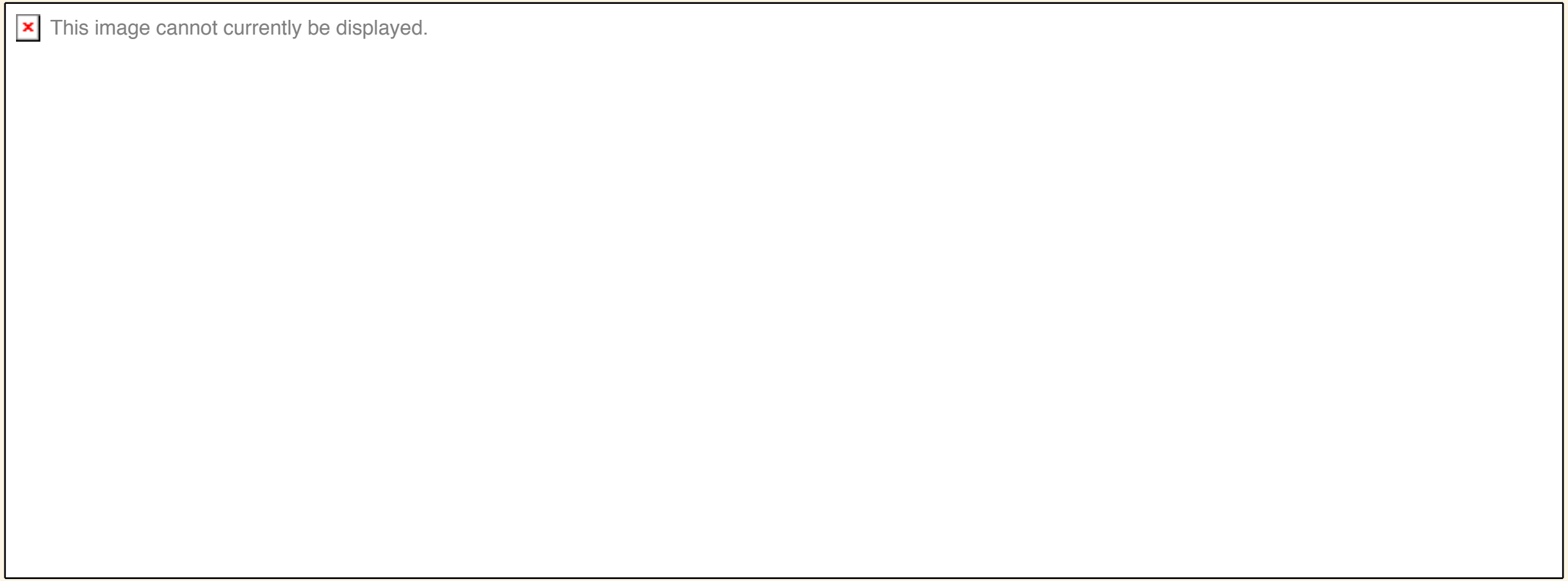
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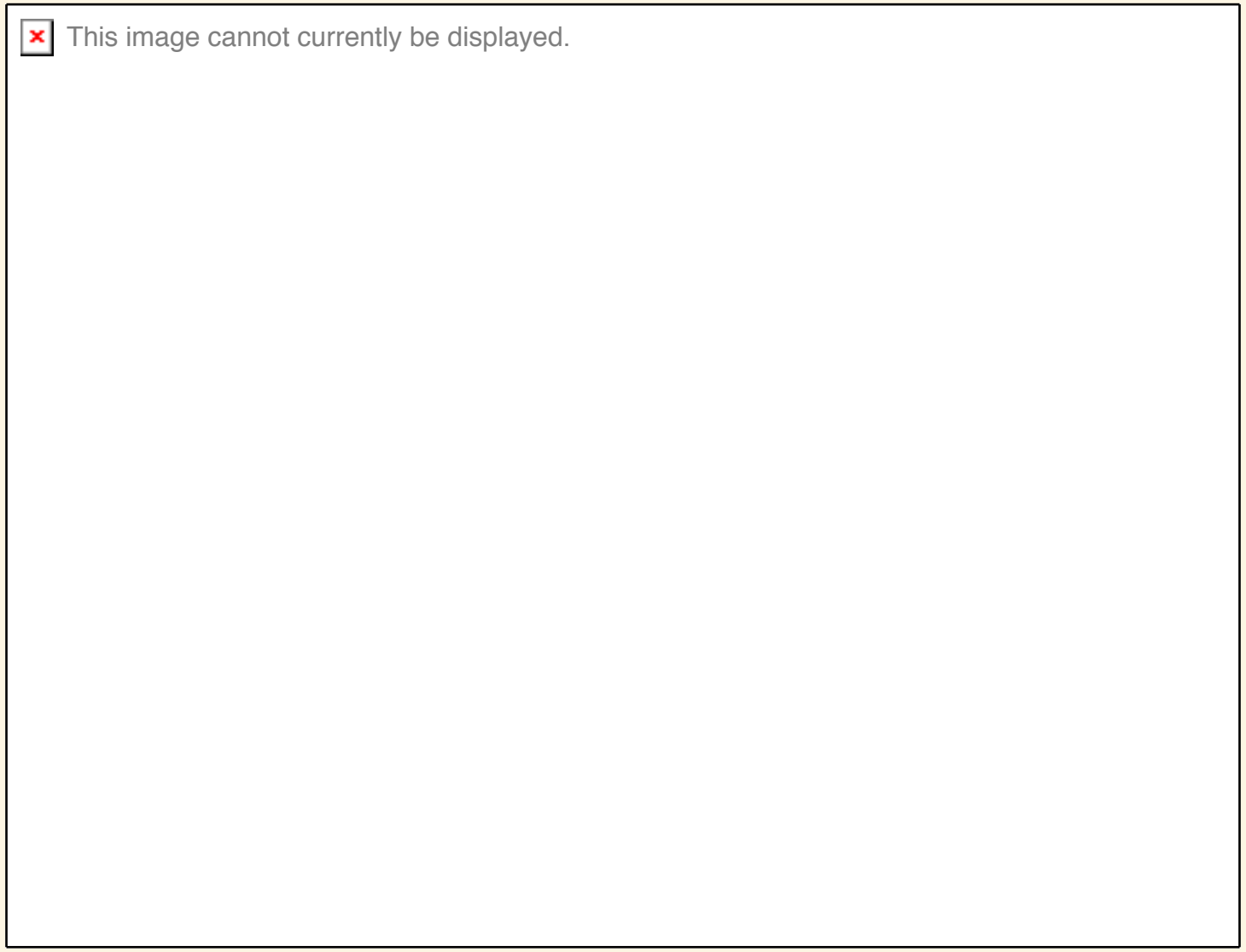
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Extending our tools to enable the analysis of **longitudinal tumor evolution**

The progression of a refractory breast cancer patient across three courses of treatment over 2.5 years, whose tumor was sequenced at 5 distinct time points, until just before death.

Mutation calling and clustering in longitudinal tumor sequencing data

B2

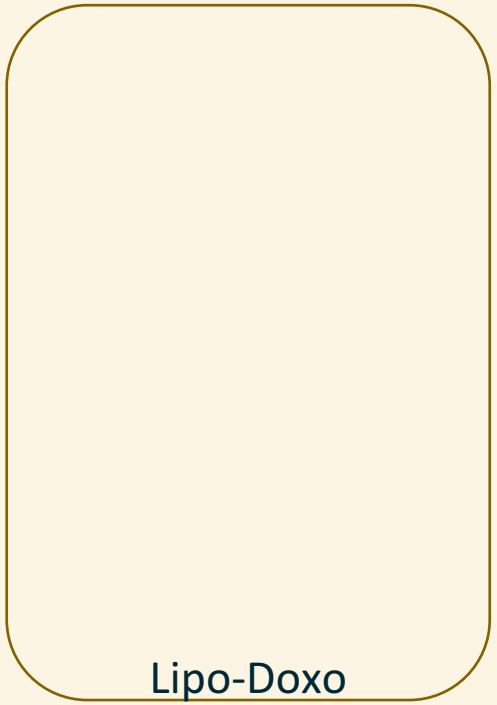
- Mutations are clustered according to their allele frequencies (cellular prevalence) at **all** sequenced time points
- All variants had to be **jointly** called across the normal control sample and the five tumor biopsies

Subclone evolution reconstructed by running SubcloneSeeker over consecutive pairs of time points

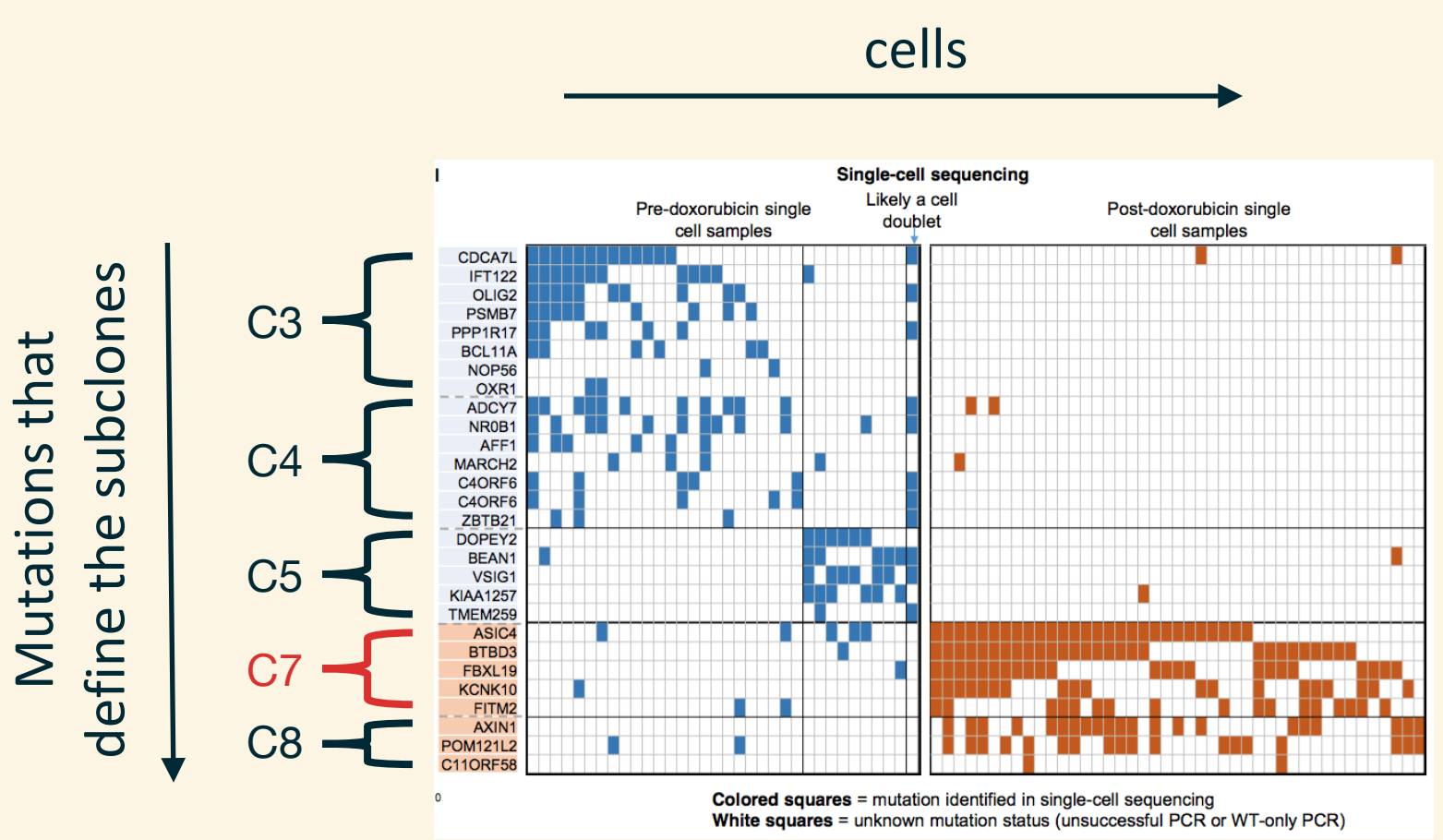
Multiple “solutions” exist

“Consensus”

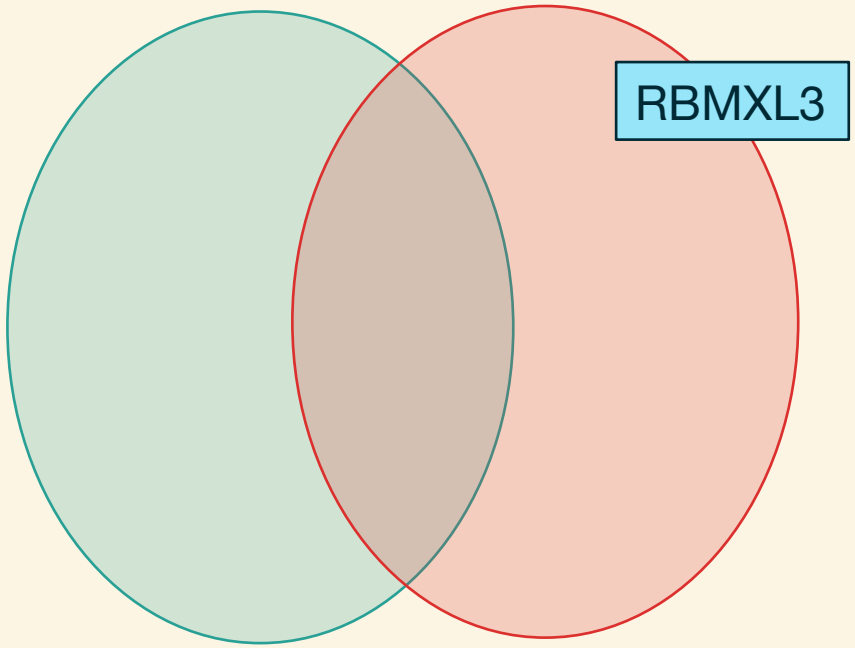
Confirming computational subclone structure prediction with single-cell genotyping



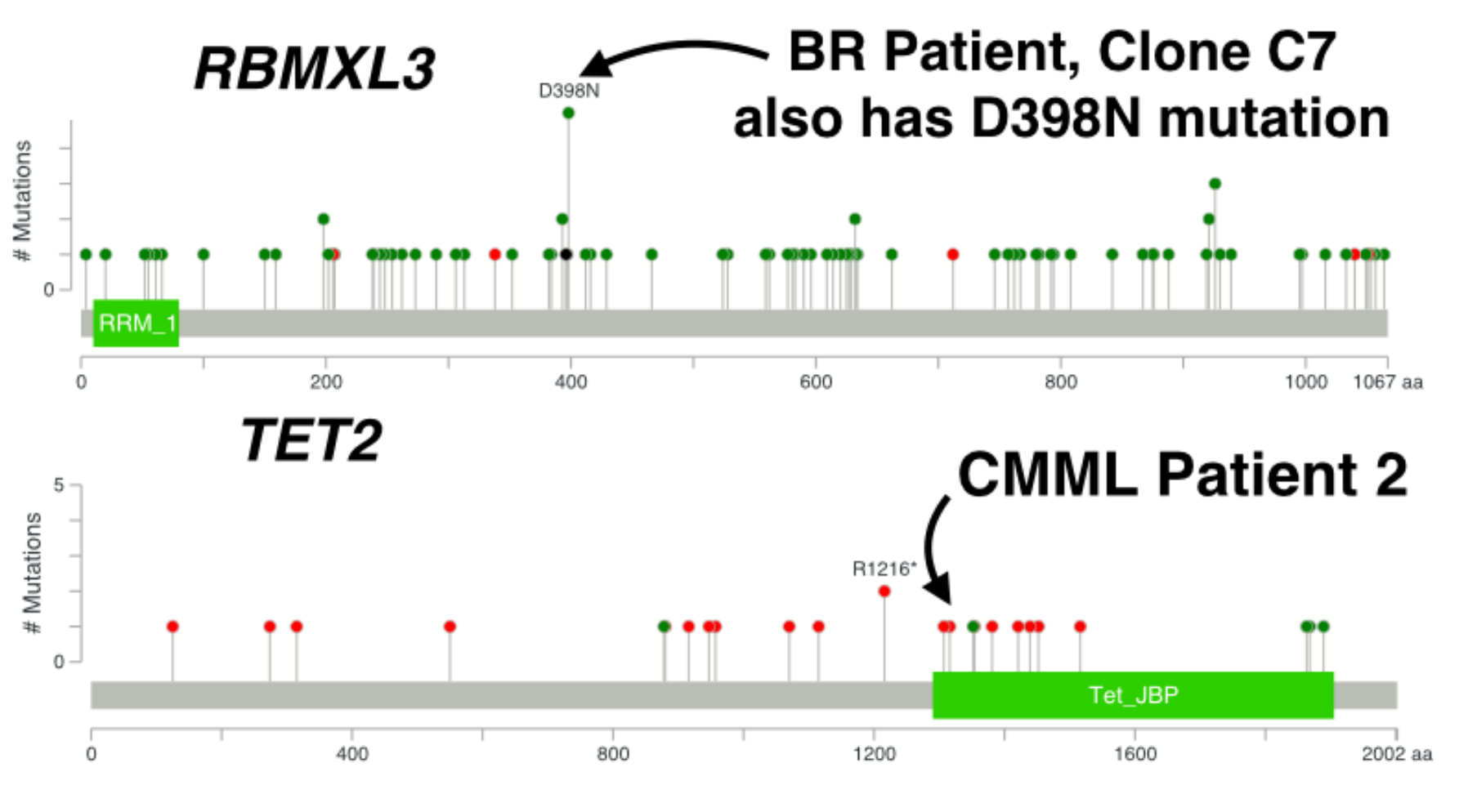
Single-cell sequencing data from **pre-Doxo** and **post-Doxo** cells, by genotyping groups of mutations that define the variant clusters, and the subclones


cells
Mutations that define the subclones
C3
C4
C5
C7
C8
Single-cell sequencing
Pre-doxorubicin single cell samples
Likely a cell doublet
Post-doxorubicin single cell samples
CDCA7L
IFT122
OLIG2
PSMB7
PPP1R17
BCL11A
NOP56
OXR1
ADCY7
NR0B1
AFF1
MARCH2
C4ORF6
C4ORF6
ZBTB21
DOPEY2
BEAN1
VSIG1
KIAA1257
TMEM259
ASIC4
BTBD3
FBXL19
KCNK10
FITM2
AXIN1
POM121L2
C11ORF58
Colored squares = mutation identified in single-cell sequencing
White squares = unknown mutation status (unsuccessful PCR or WT-only PCR)

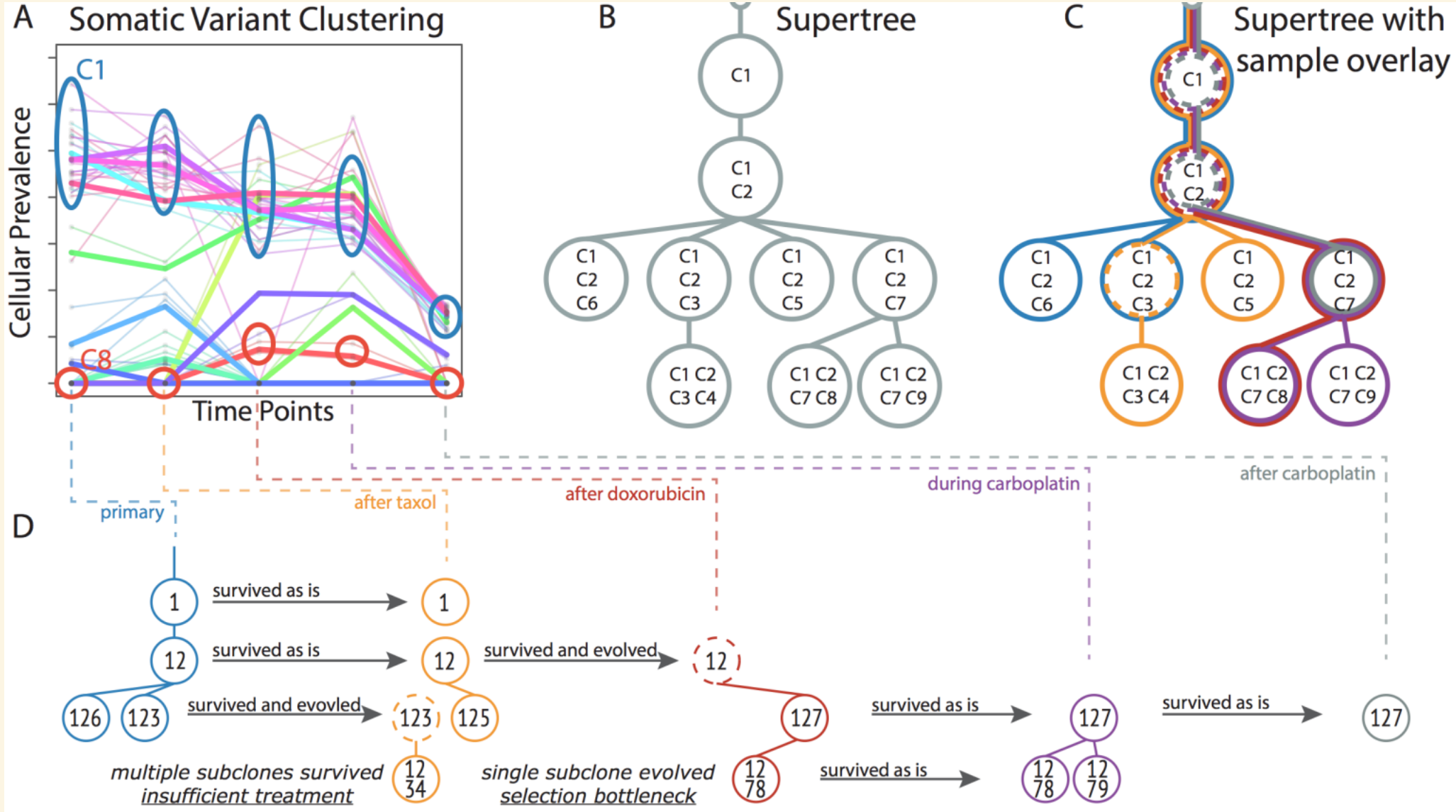
Interpreting subclone evolution in the context of disease progression



Variant interpretation

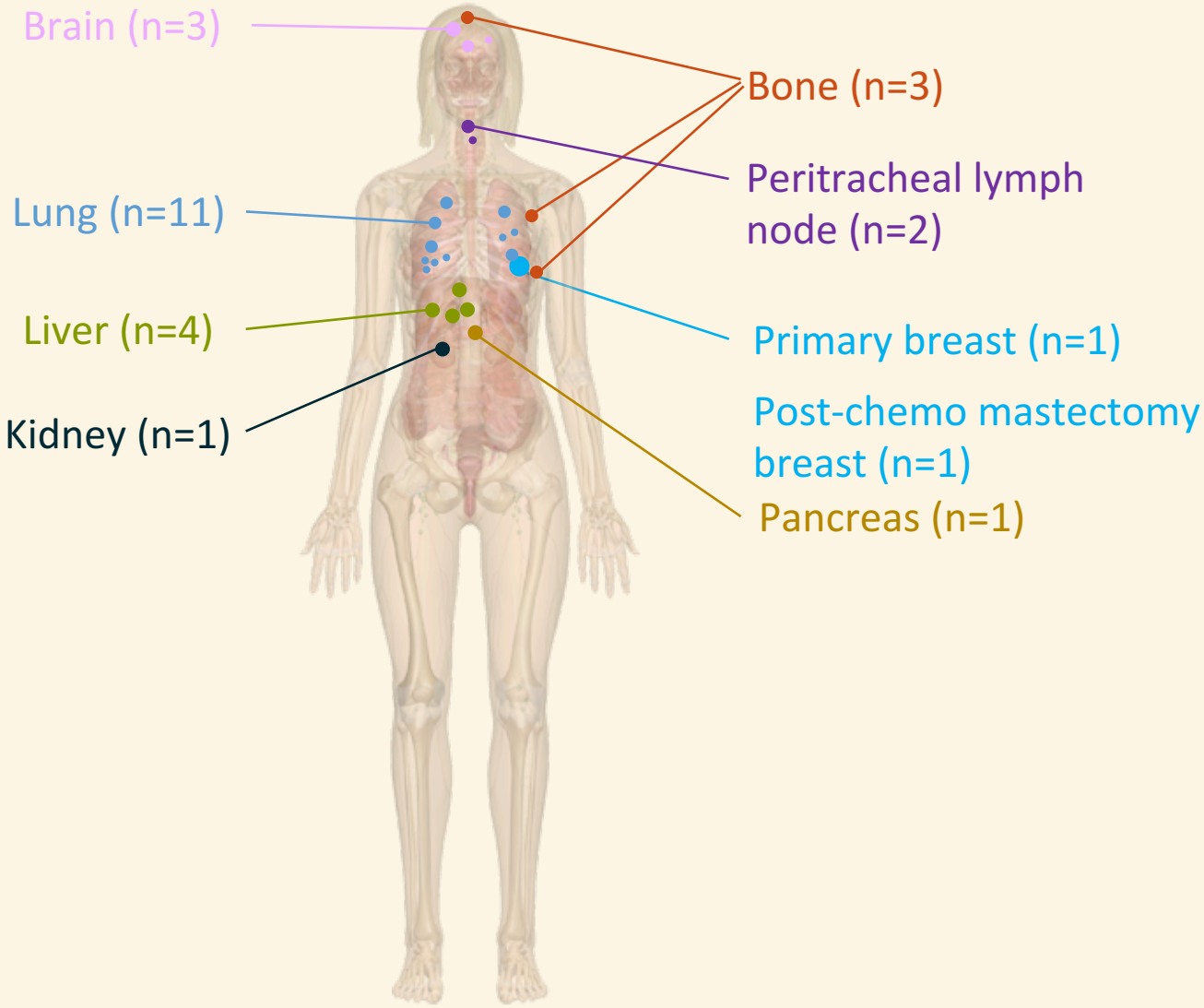


Current focus is on developing a general algorithmic solution for reconstructing subclone structure in a longitudinal setting (SuperSeeker)



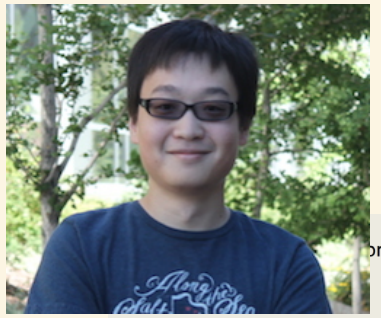
Our “driver” dataset is from a “rapid autopsy” metastatic breast cancer patient

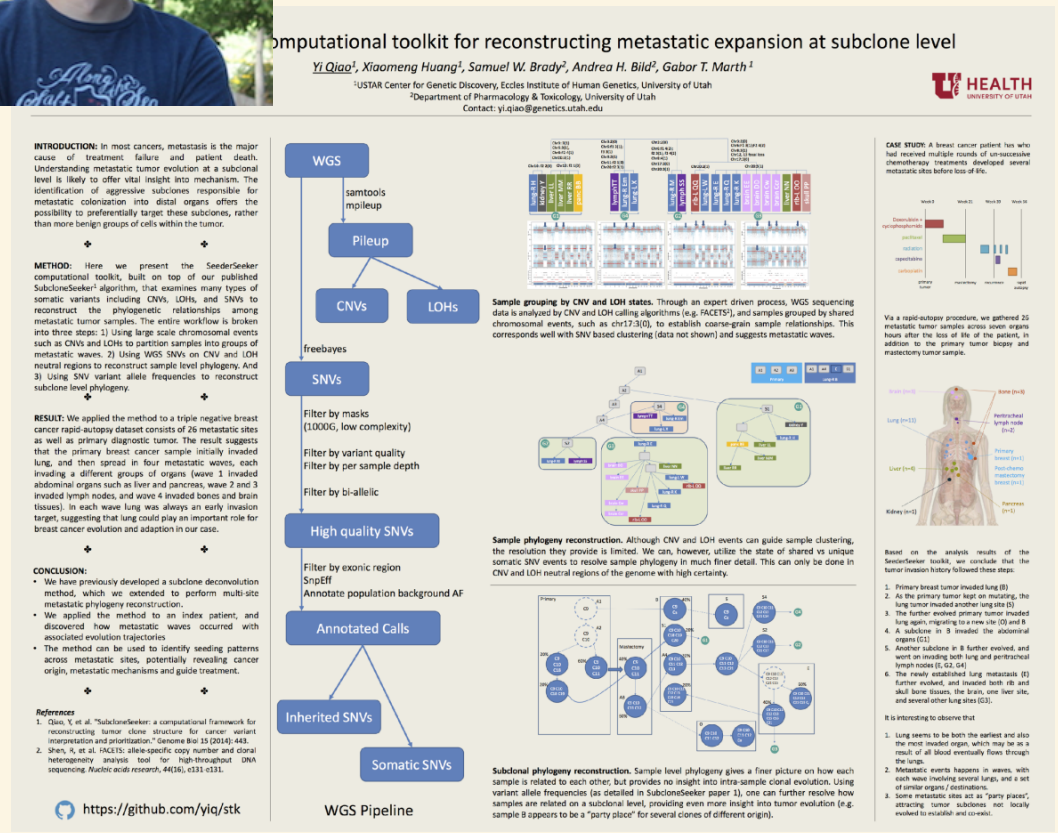
- Triple-negative disease
  - Primary biopsy at diagnosis
  - Mastectomy at recurrence
  - 26 metastases from various organs removed during *rapid autopsy,* hours after death
  - 2 normal control samples (skin)
  - **30 biopsies across 8 organs**
- 60X WGS + WES Illumina sequencing data collected at Wash U for all biopsies + 2 normal controls from skin + 2 primary tumors
- Inherited variants in BRCA2, not in BRCA1
- All tumor sites share somatic homozygous TP53 missense SNV and RB loss



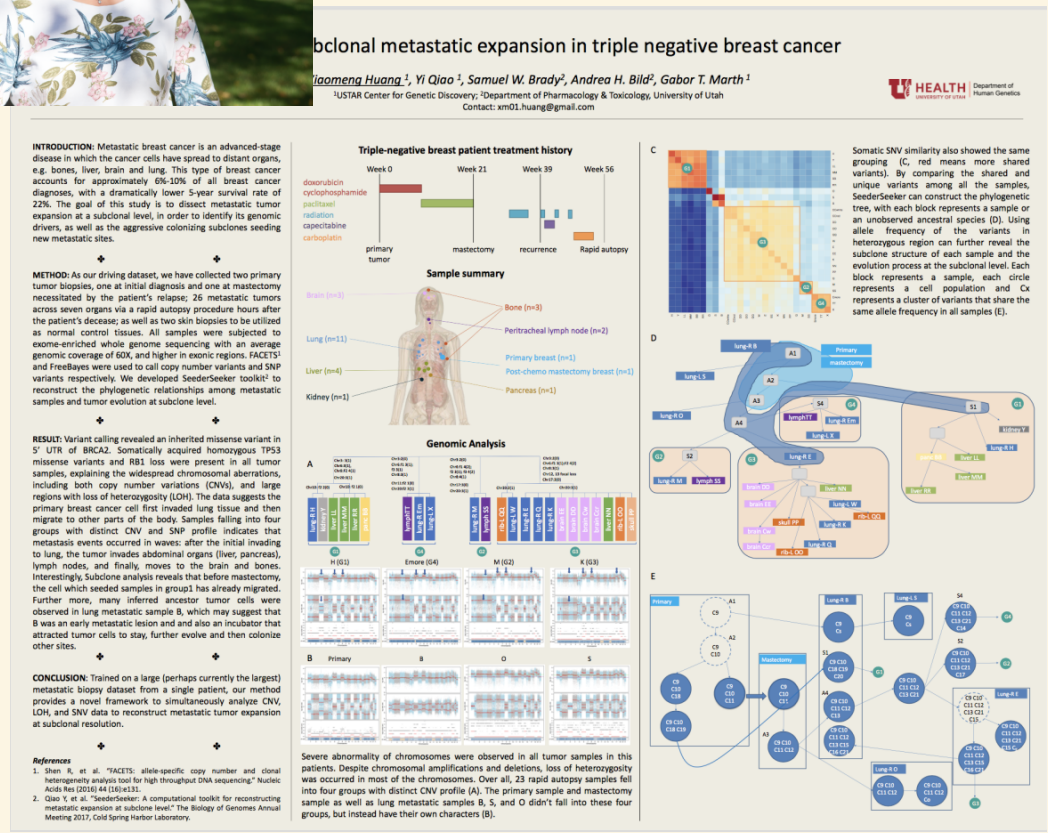
Please visit our posters to see the current state of this analysis

Yi Qiao





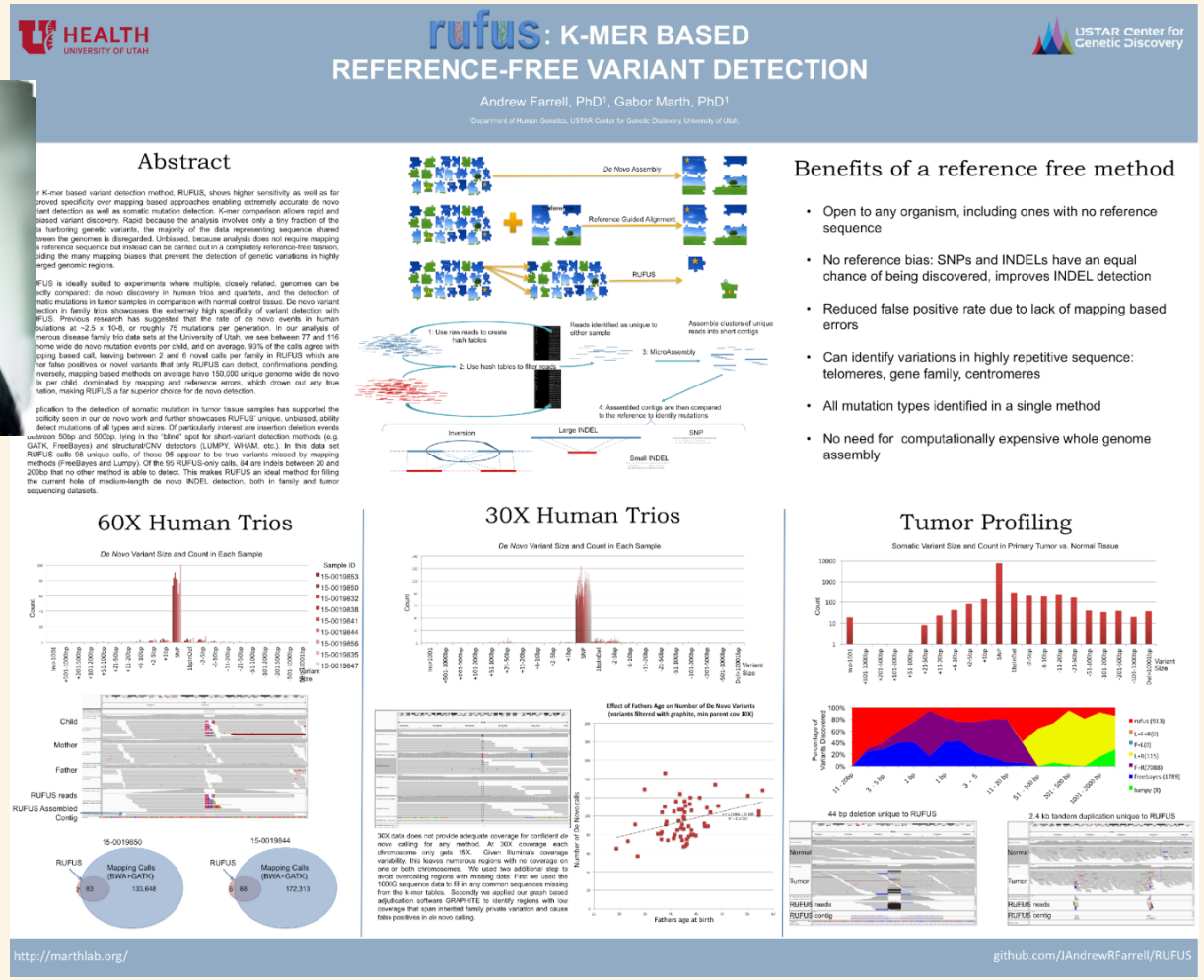
Xiaomeng Huang



Ongoing

- Novel, reference-free methods for somatic mutation identification

Andrew Farrell



Ongoing

- Cancer mutation annotation
  - Set-aside project to integrate with CiViC (Obi and Malachi Griffith)
- Current collaborations
  - Refractory, triple-negative, and metastatic breast and ovarian cancers (Andrea Bild, David Bowtell, Lynn Henry)
  - Hematological malignancies (Michael Deininger, Debbie Stephens, John Byrd)
  - Patient-derived tumor models (Bryan + Alana Welm, K.T. Varley, Jay Gertz)
- Subclone analysis using single-cell sequencing data
  - **Looking for SC collaborations!**

Funding

National Human Genome Research Institute
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R41HG009096
R01HG009000 pending

National Cancer Institute
U24CA209999

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USTAR

SFARI Simons Foundation Autism Research Initiative

The Eric S. Margolis Family Foundation

Team

- PIs: Aaron Quinlan and Gabor Marth (contact)
- Clinical oncology: Theresa Warner (breast, ovarian); Michael Deininger (hematological)
- Experimental/laboratory: Andrea Bild, Samuel Brady
- Computational: Ryan Layer, Andrew Farrell, Dillon Lee (detection); Yi Qiao, Xiaomeng Huang (subclone analysis); Brent Pederson (annotation); Tonya DiSera, Chase Miller (web tools)



Ad

Postdoc positions in computational cancer analysis tool development available.

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