Genomic characterization of brain metastases and paired primary tumors reveals branched evolution and potential therapeutic targets

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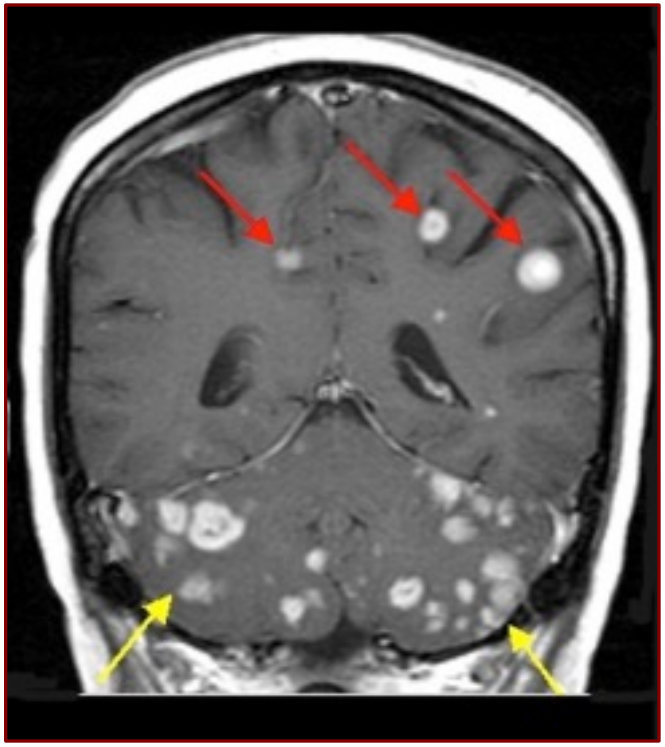
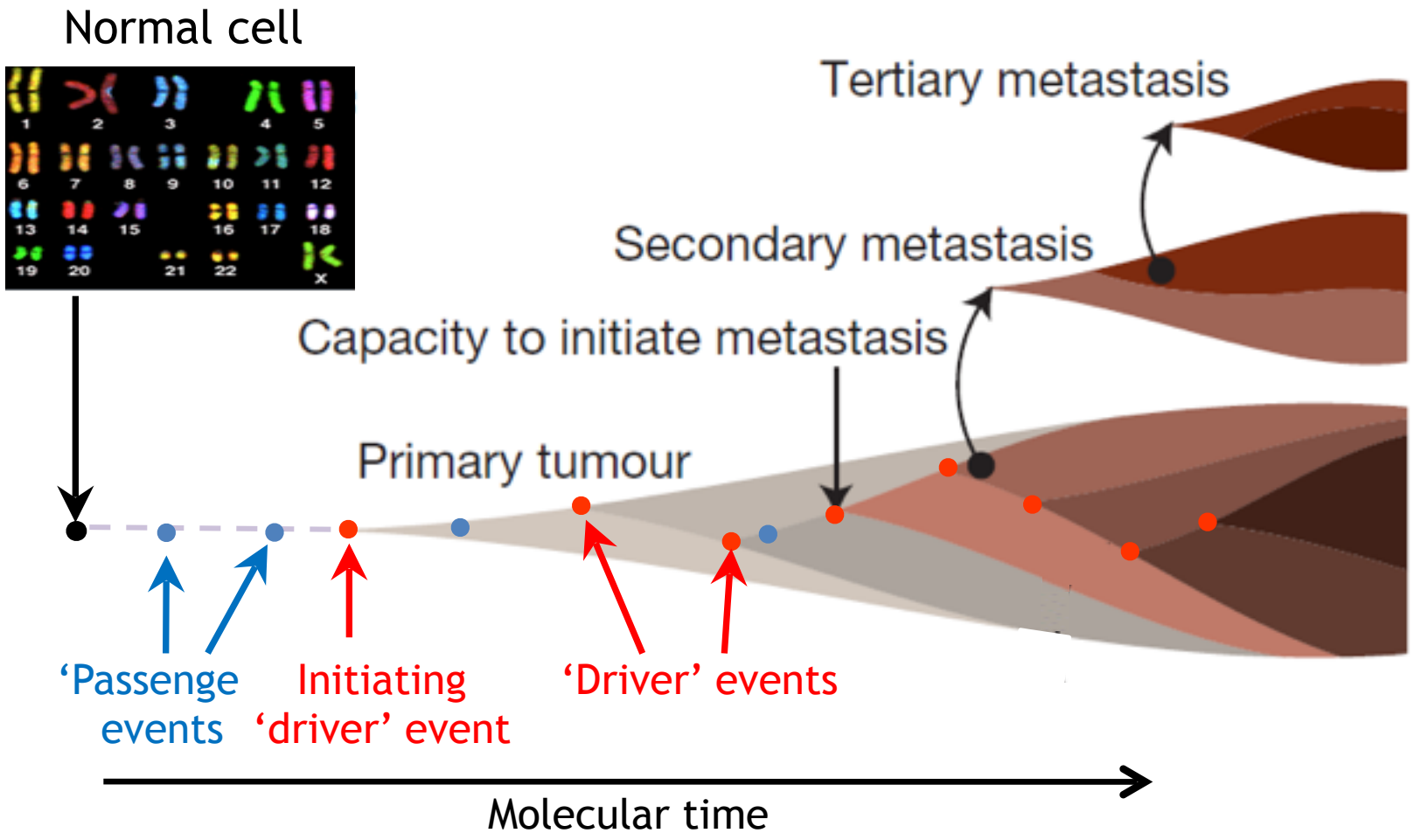
Brain metastases are an unmet clinical need



- Most common malignancy of the brain
- Up to 25% of cancer patients
- Most derived from lung, breast, melanoma
- Incidence is rising as systemic therapies are improving.

We have a limited understanding of how brain metastases genetically evolve from their primary tumor

Clonal theory of somatic cancer evolution

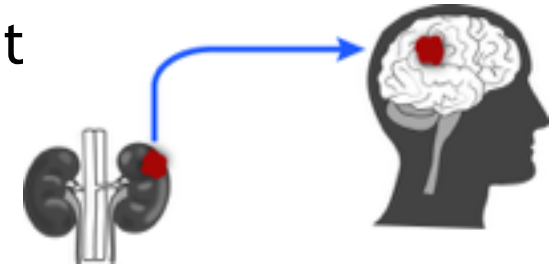


Adapted from Campbell et al. *Nature* 2010

Models of metastasis evolution

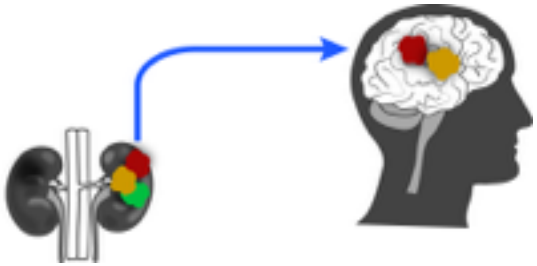
1. Historical dogma: Metastasis arises from single clone

- Single clone seeding of metastatic deposit
  - *Science* 1982, Talmadge et al.
  - *Cancer Res* 1986, Fidler and Talmadge



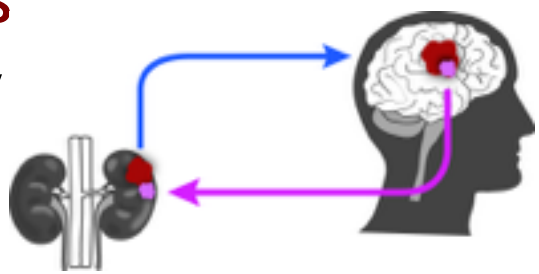
2. Polyclonal spread of metastasis

Multiple subclones
contribute to a single metastasis
- *Cancer Res 1982*, Poste et al.
- *Nature 2015*, Gundem et al.

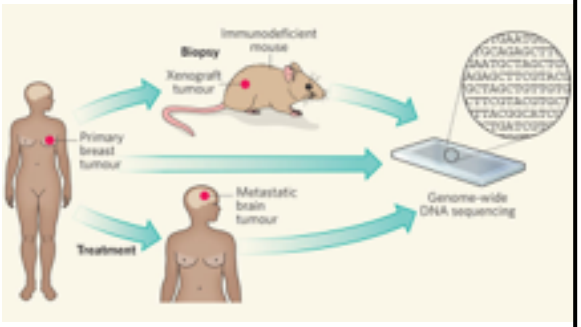


3. Tumor self-seeding from metastasis

Cells from metastasis re-seed the primary
- *Cell* 2009, Kim and Massagué

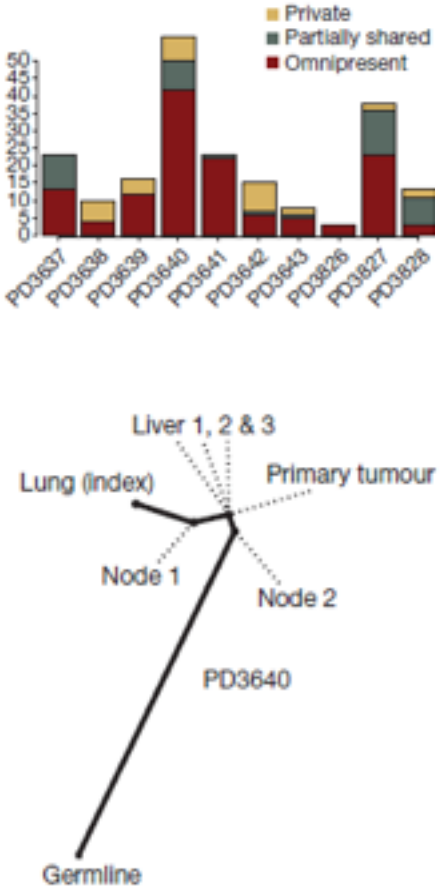


Clonal evolution in the metastatic process



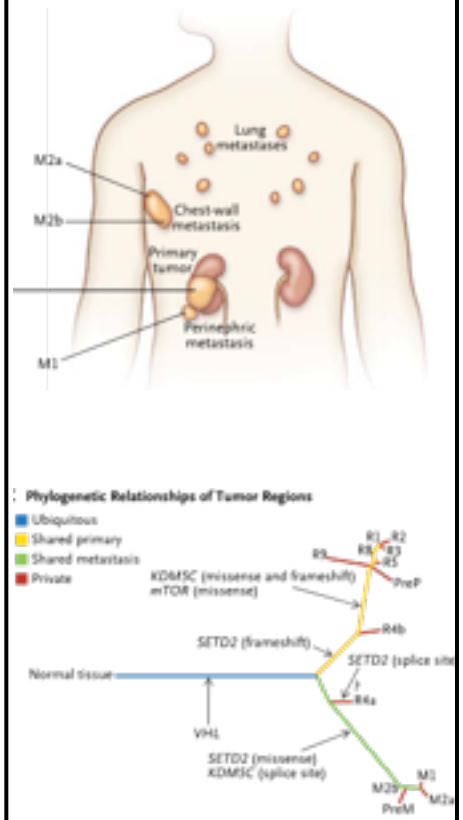
Few *de novo* mutations in brain metastasis (n = 1)

Ding et al, *Nature* 2010



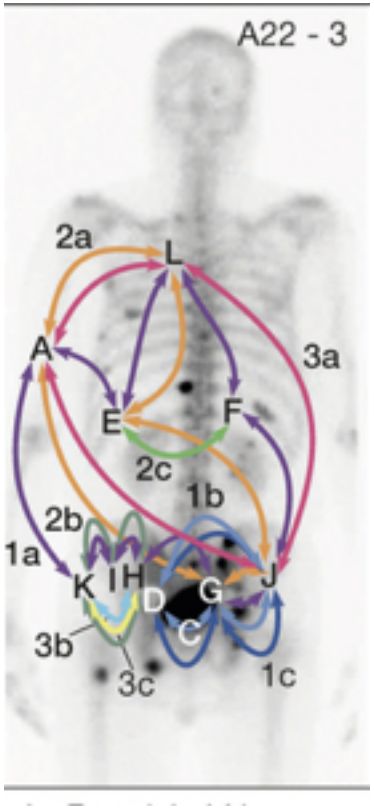
Organ-specific branches

Campbell et al., *Nature* 2010



Intratumoral heterogeneity

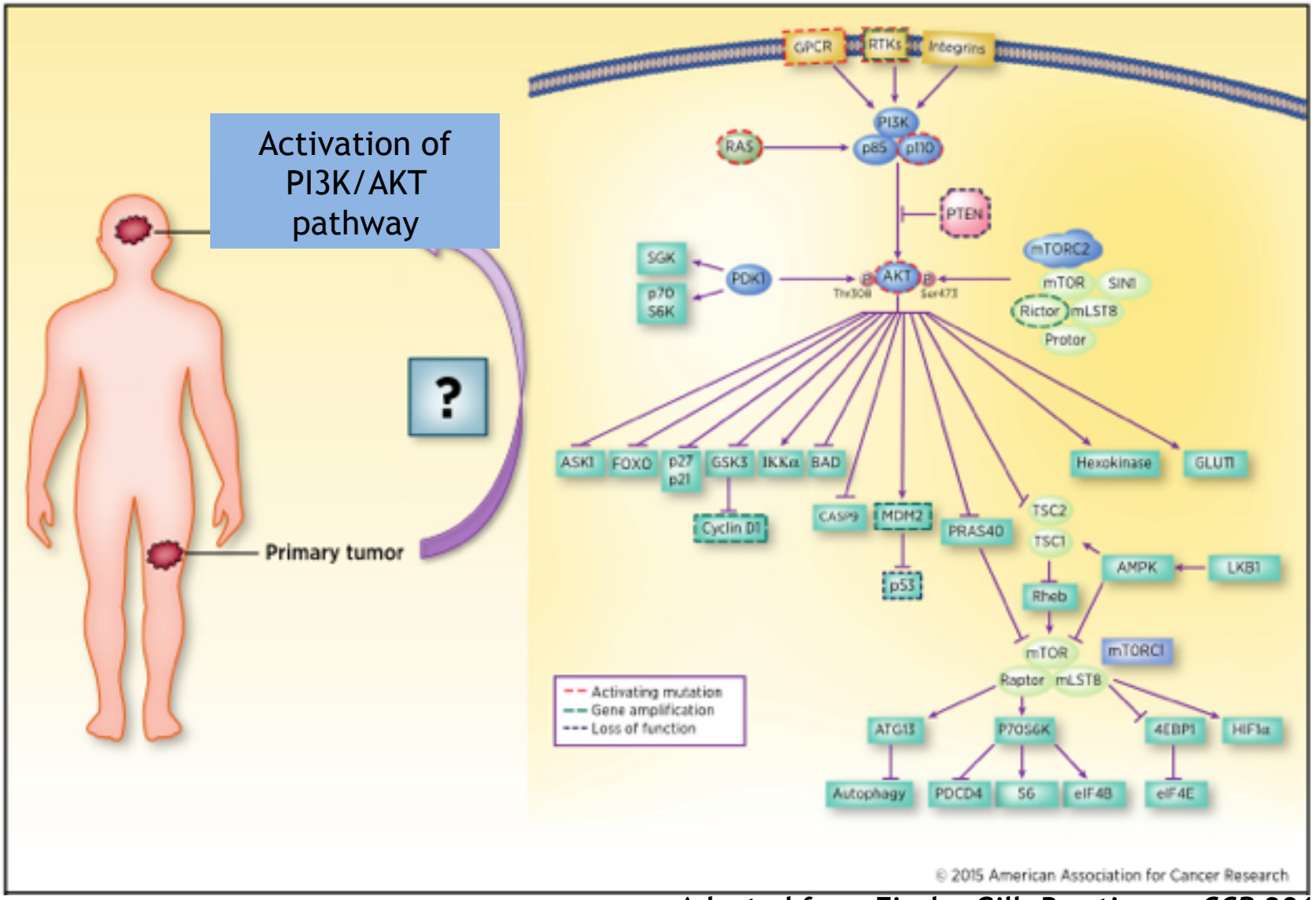
Gerlinger et al., *NEJM* 2012



Metastasis to metastasis spread

Gundem et al. *Nature* 201

Prior data suggests activation of PI3K pathway in brain metastases



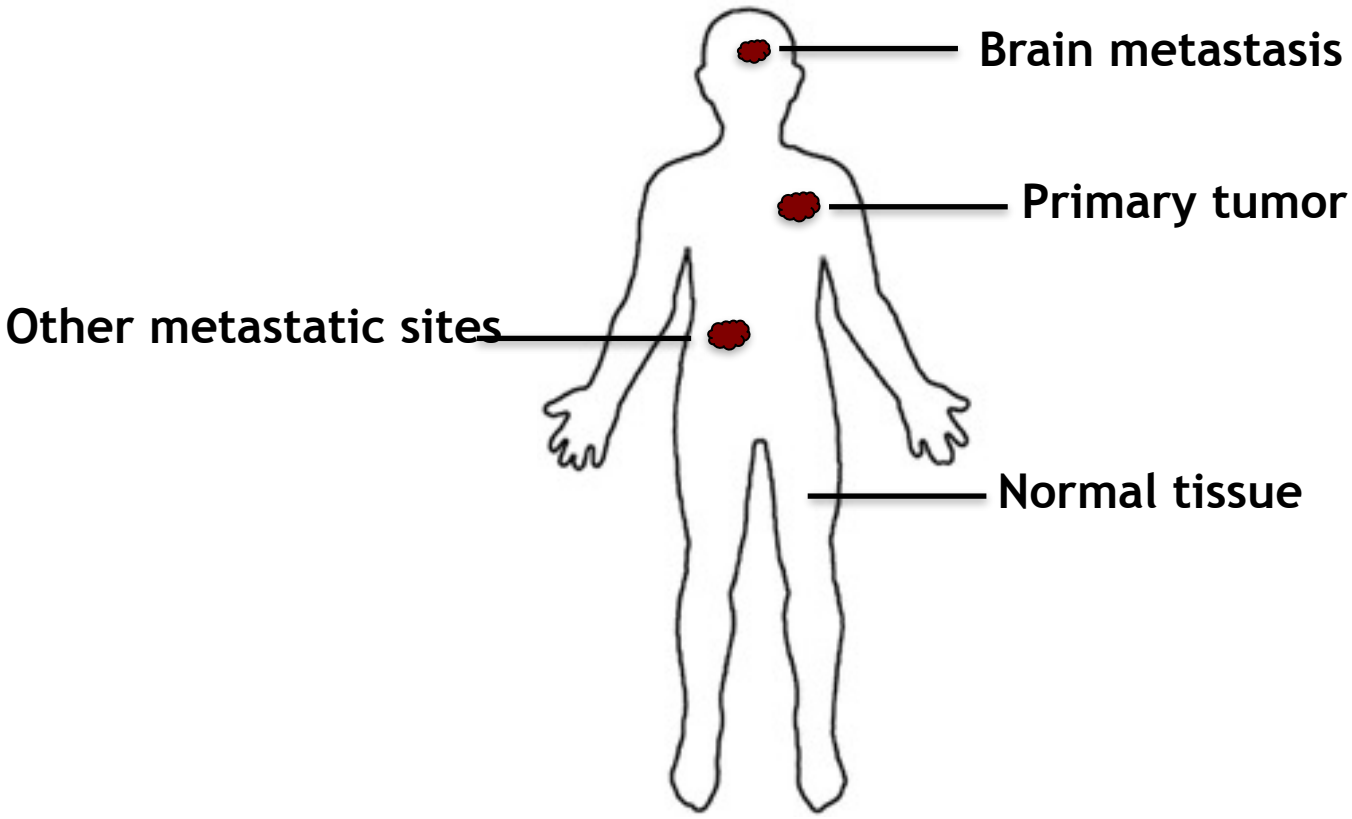
Adapted from Eisele, Gill, Brastianos, *CCR* 2015

Objectives

- To elucidate the evolutionary patterns leading to brain metastases.
- To identify whether brain metastases harbor clinically significant genetic differences compared to their primary tumors.
- To determine the extent to which clinically significant mutations are shared among regionally, anatomically and temporally distinct brain metastasis sites.
- To determine whether lymph nodes or extracranial metastases are genetically similar to brain metastases and might serve as their proxy for clinical decision making

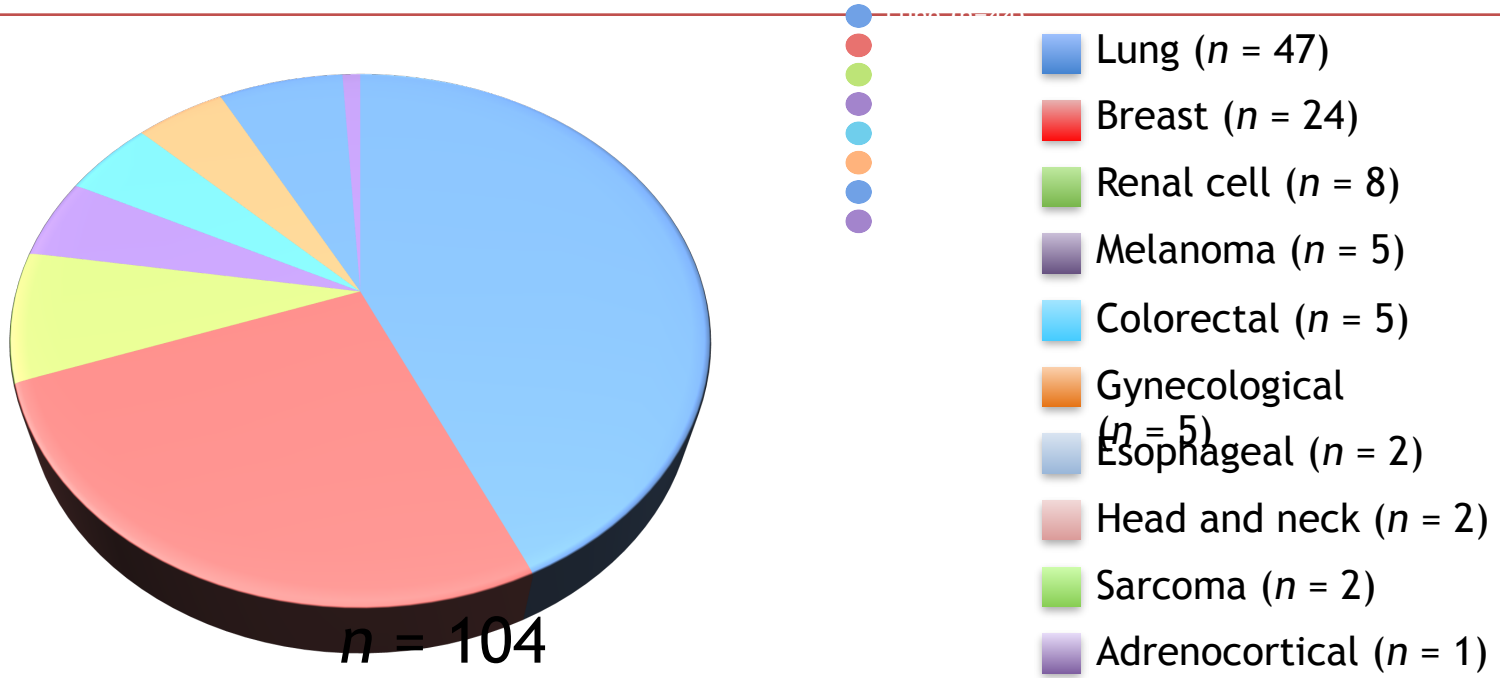
Study design

- Whole-exome sequencing of 104 brain metastases matched with primary and normal tissue
- Including 15 with additional extracranial sites or temporally/ regionally/anatomically separated brain metastases



Cohort summary of sequenced patients

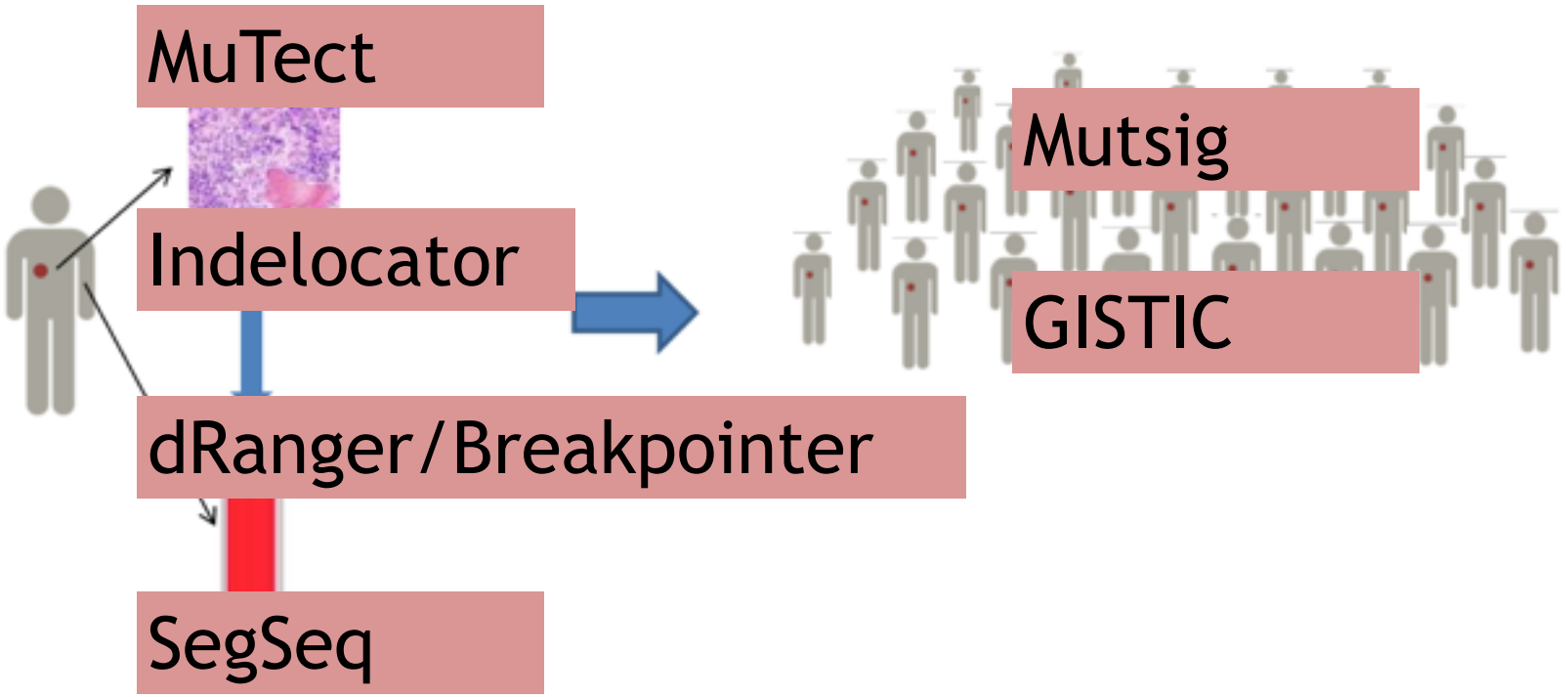
Cohort	
Age of diagnosis of primary tumor (yrs)	56 (44-68)
Gender	67 F / 34 M
Median time to diagnosis of brain metastasis (yrs)	2 (0-11)
Median # treatment regimens between primary and brain metastasis	1 (0-10)



Identifying candidate driver genes

Characterization (Individual)

Interpretation (Population)

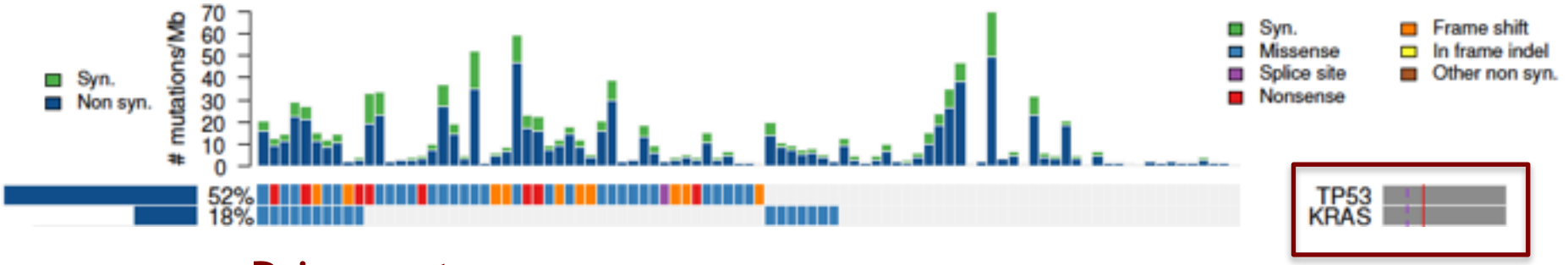


What is the full set of genome alterations within the cancer (and germ-line)—mutations, copy number, translocations, etc?

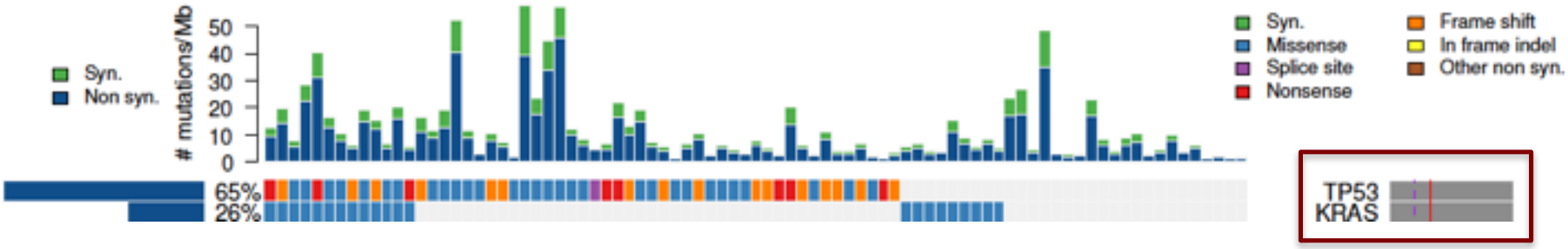
(1) Which genome alterations are **statistically significant** in the population?
(2) In which **genes** and **pathways** do these alterations occur?

Adapted from Gad Getz

TP53 and KRAS are top hits in metastases and primary tumors



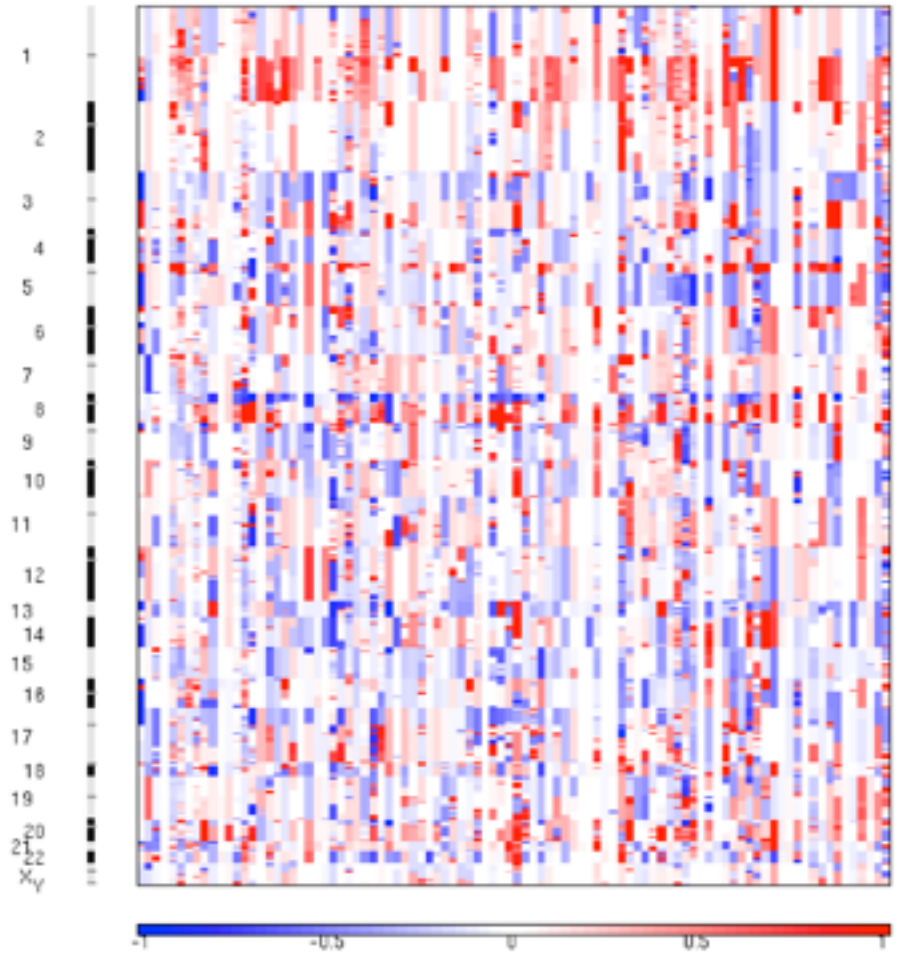
Primary tumors



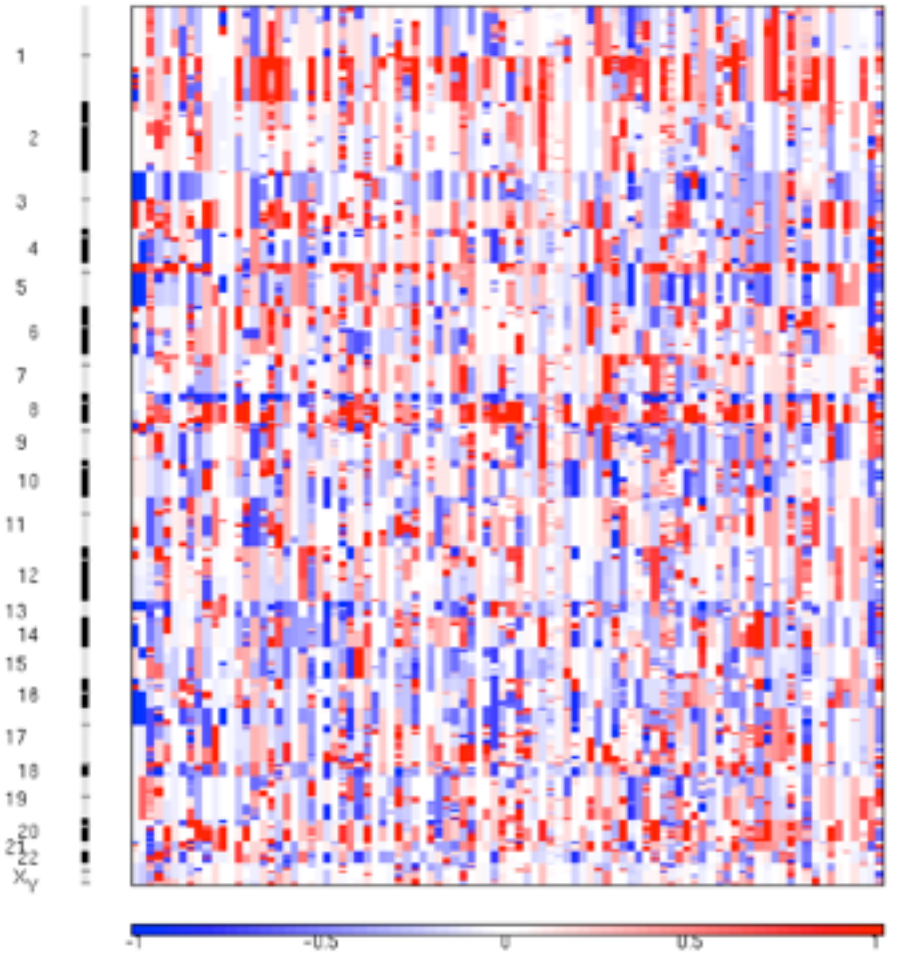
Brain metastases

Copy number profiles are complex

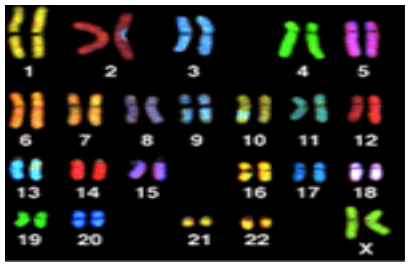
Primaries

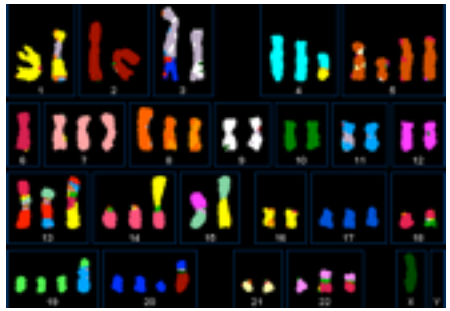


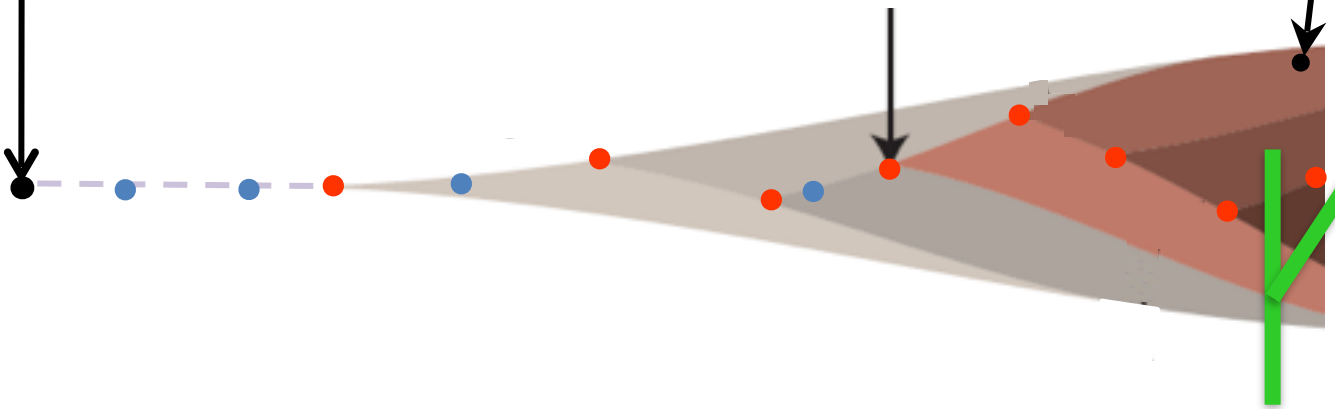
Mets

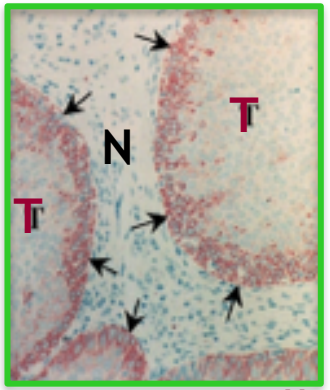


Cancer tissue samples are heterogeneous

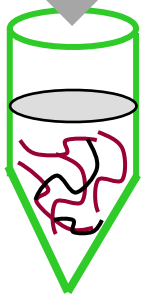








T = Tumor cells
N = Normal cells



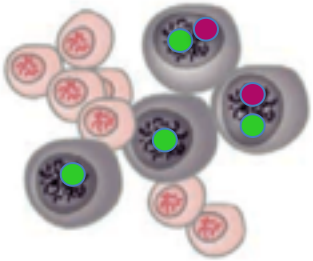
Mixed DNA aliquot

Cancer tissue sample
- Heterogeneous mixture of normal cells and related cancer clones

Can we deconvolve cancer tissue samples?

Sequencing of cancer-tissue derived DNA aliquot

Copies per *cancer* cell for each *somatic* mutation

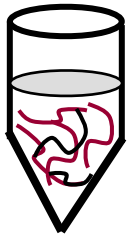


- Clonal mutation: present in all *cancer* cells sequenced
- Subclonal mutation: present in a subset of cancer cells sequenced

Carter et al., *Nat. Biotech* 2012

ABSOLUTE: statistical deconvolution of genetic heterogeneity in cancer tissue samples

Sequencing of cancer-tissue derived DNA aliquot

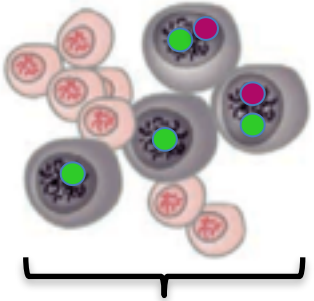


Relative abundance of variants

ABSOLUTE

Correct for purity & ploidy

Copies per *cancer* cell for each *somatic* mutation



Clonal CCF = 1.0

Subclonal CCF = 0.5

For each somatic mutation, we estimate its **cancer cell fraction** (CCF)

Gad Getz Scott L. Carter

Carter et al., *Nat. Biotech* 2012
Landau, Carter et al., *Leukemia* 2013

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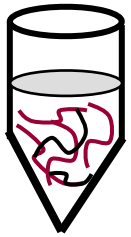

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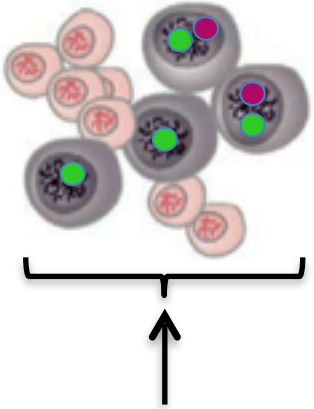


Clonal CCF = 1.0

Subclonal CCF = 0.5

For each somatic mutation, we estimate its **cancer cell fraction** (CCF)

Clonal mutations:

Affecting all cells in biopsy;

- Founder and earlier events

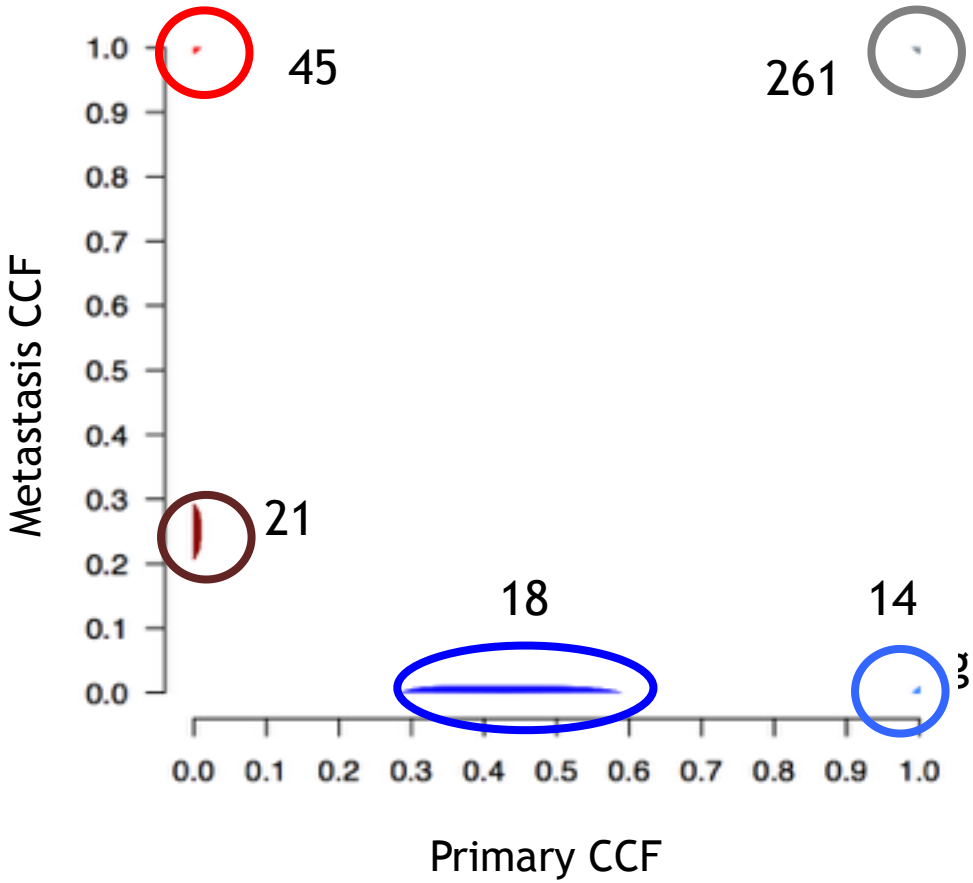
Subclonal mutations:

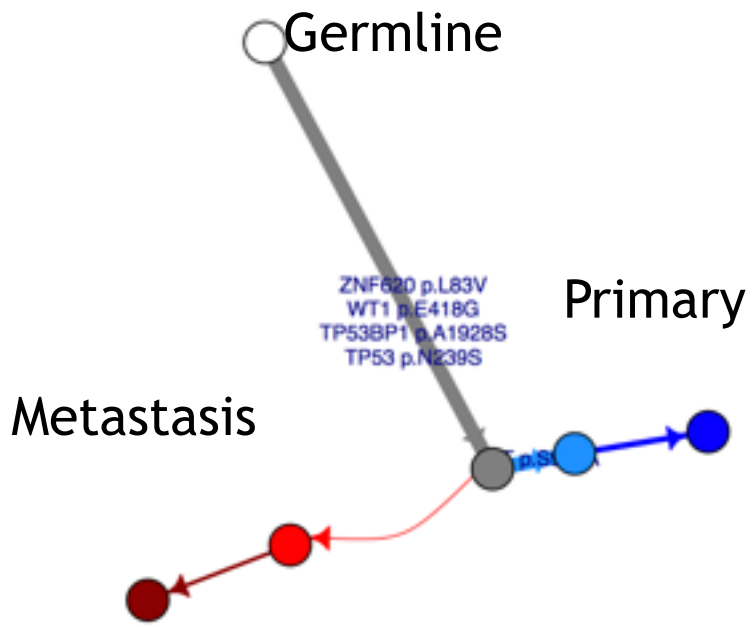
Affecting a subpopulation;

- Later events

Carter et al., *Nat. Biotech* 2012
Landau, Carter et al., *Leukemia* 2013

Phylogenetic reconstruction of a metastatic esophageal carcinoma

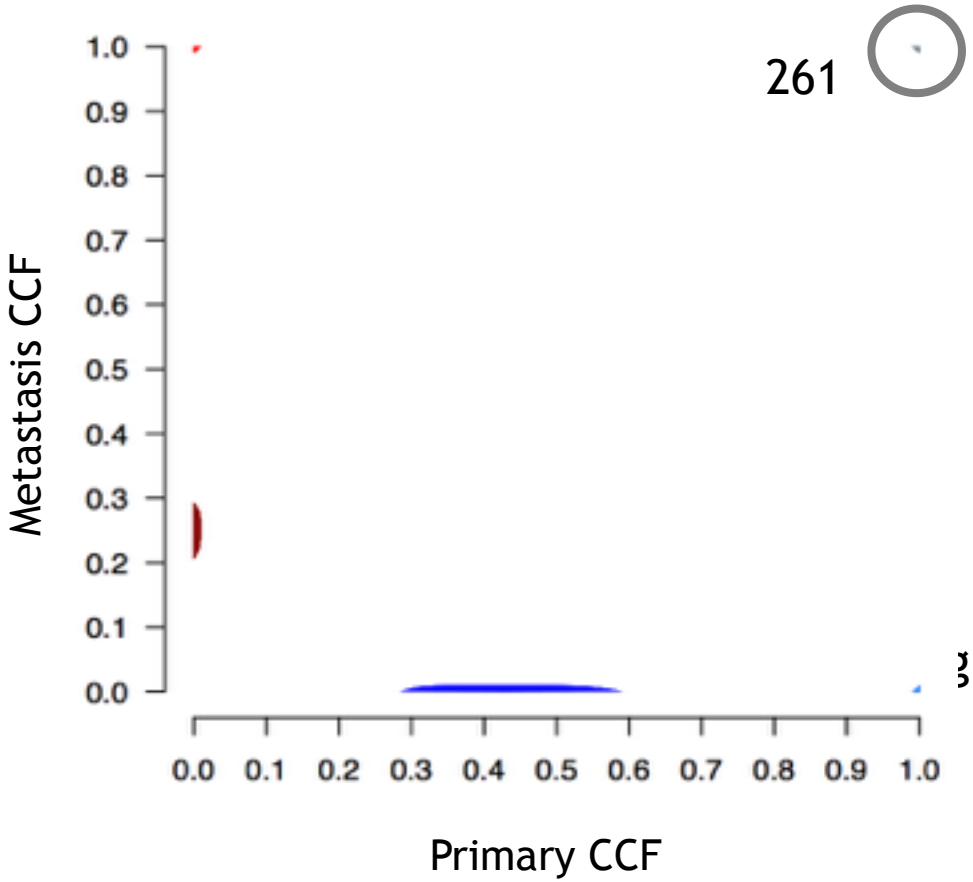


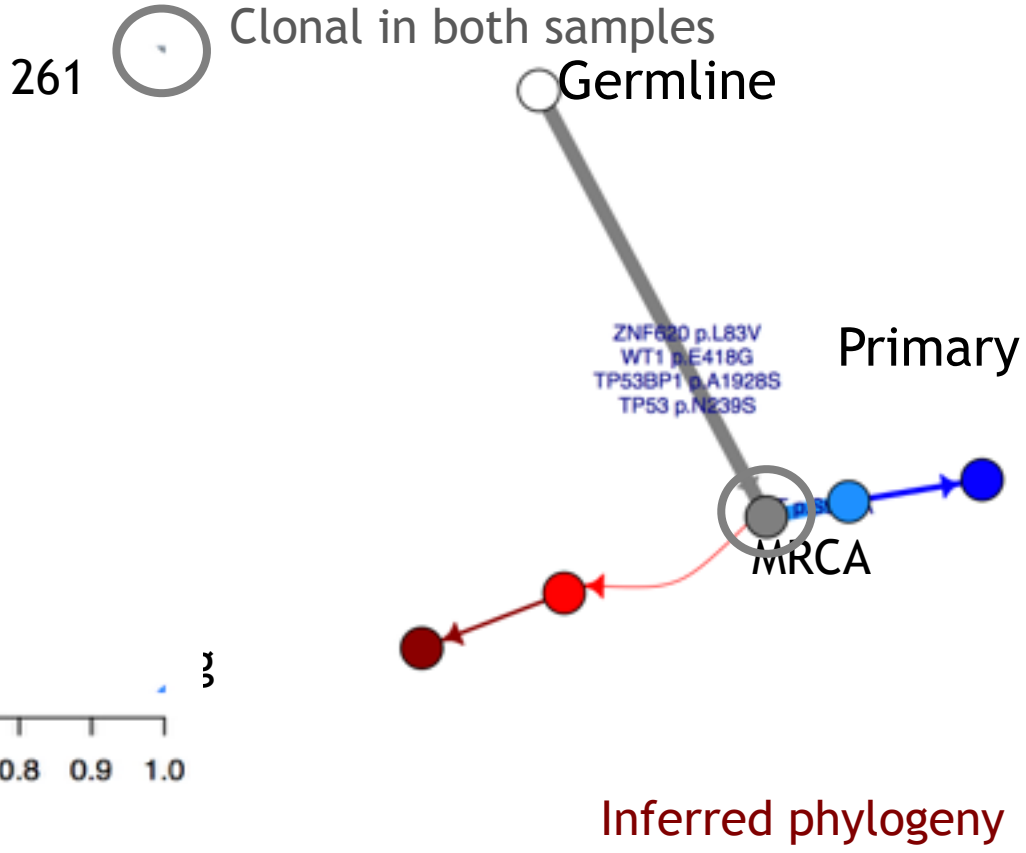


Inferred phylogeny

Brastianos, Carter et al., *Cancer Discovery 2015*

Phylogenetic reconstruction of a metastatic esophageal carcinoma





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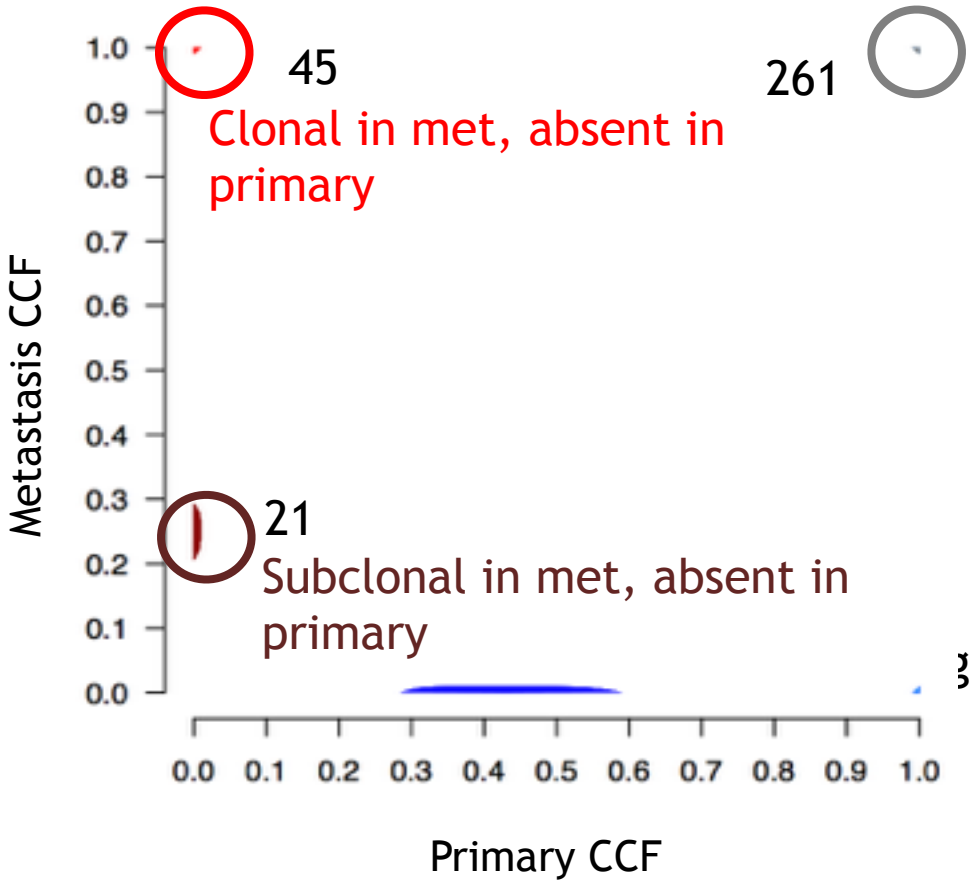

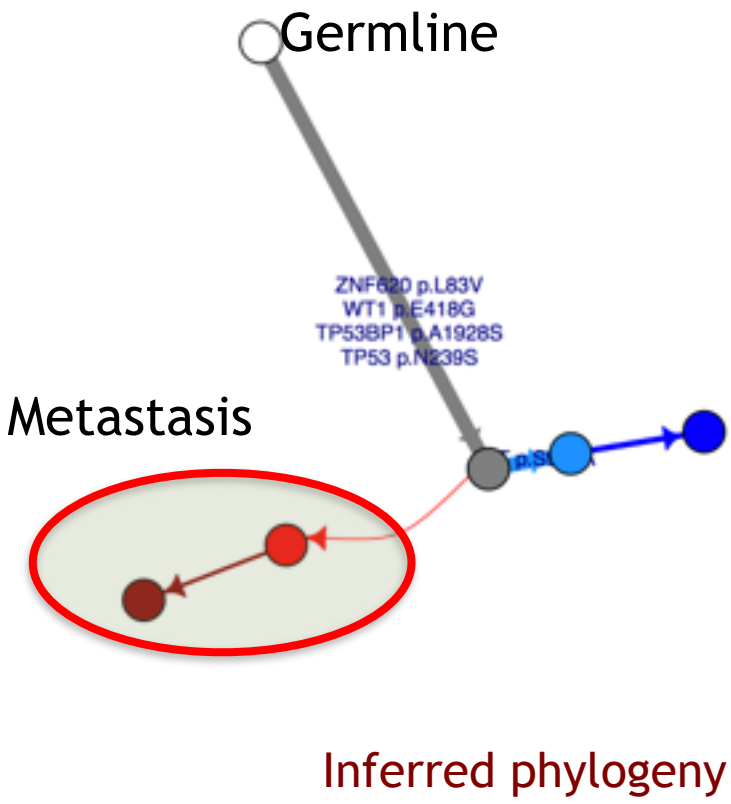

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Brastianos, Carter et al., *Cancer Discovery 2015*

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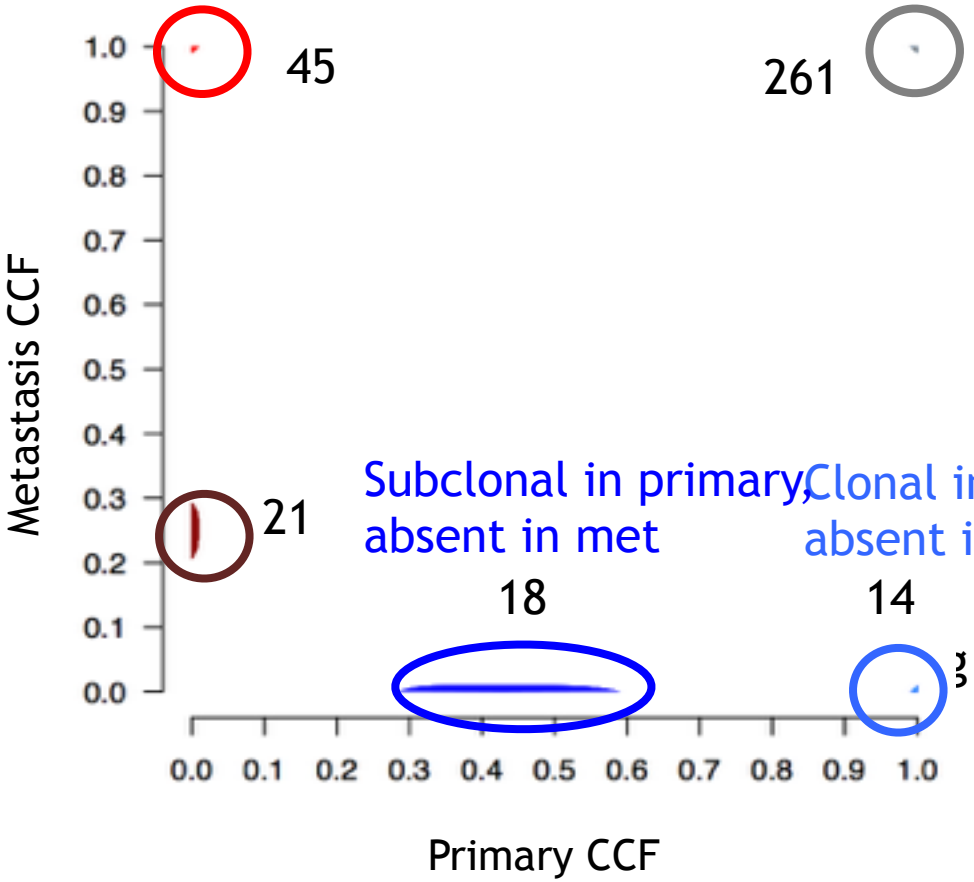

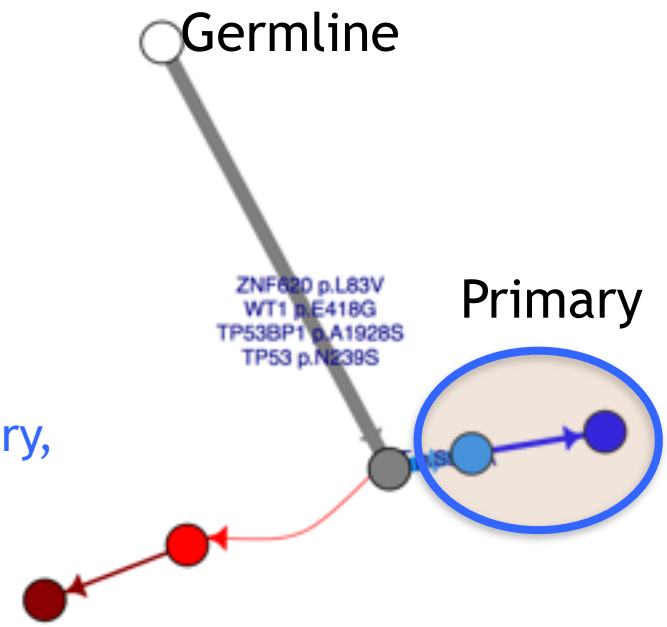

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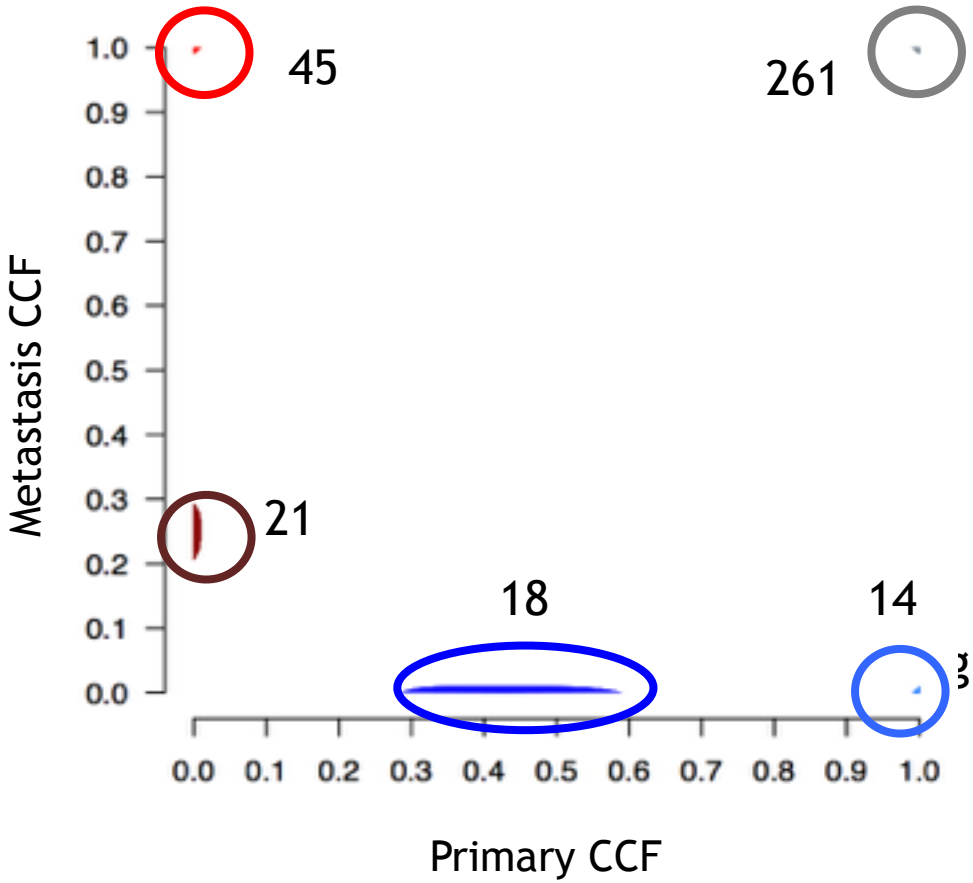
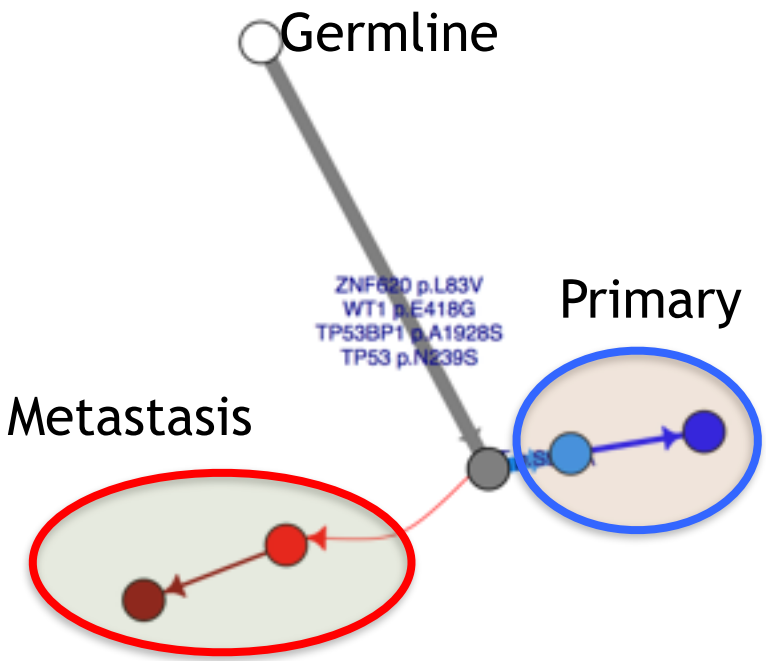


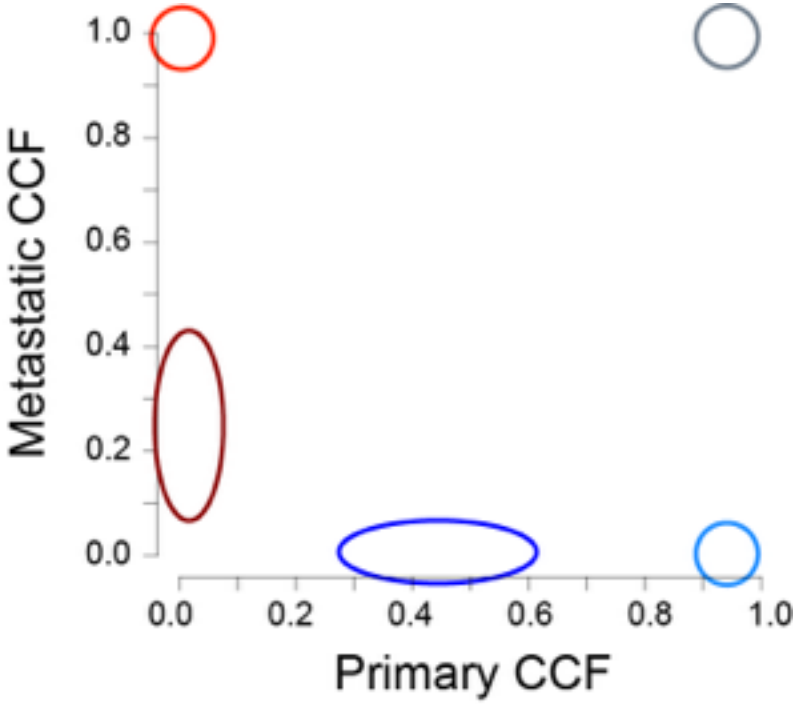


Inferred phylogeny

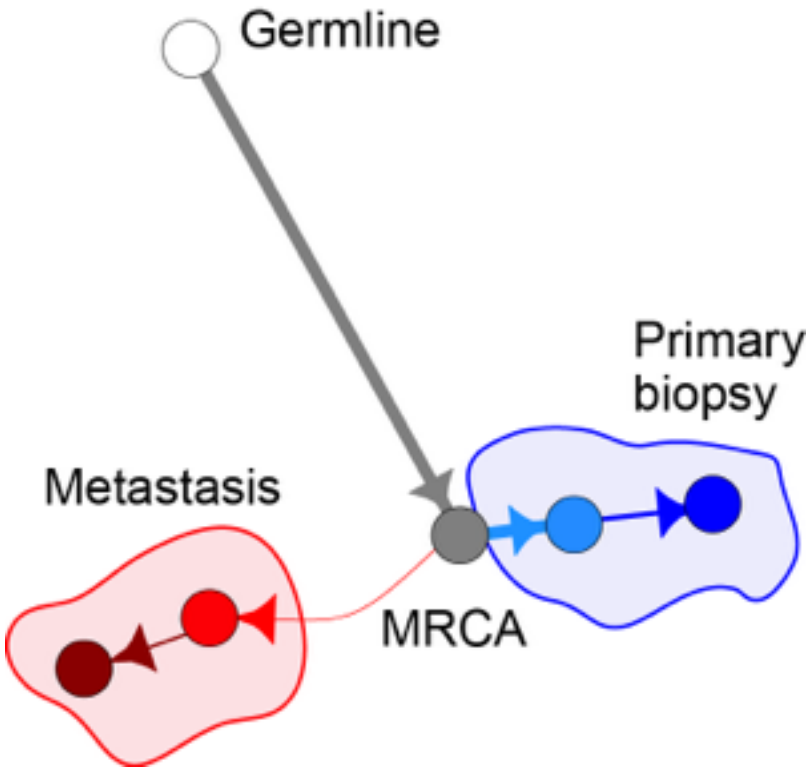
Single clonal origin of metastasis with branched evolution

Brastianos, Carter et al., *Cancer Discovery 2015*

All related samples: single clone origin with branched (sibling) relationship



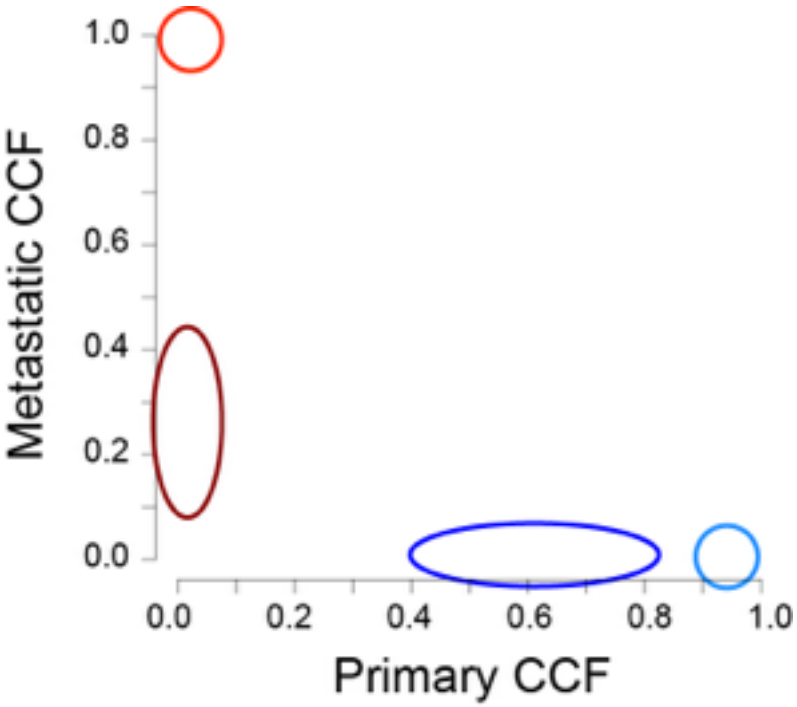
Modeled mutation data

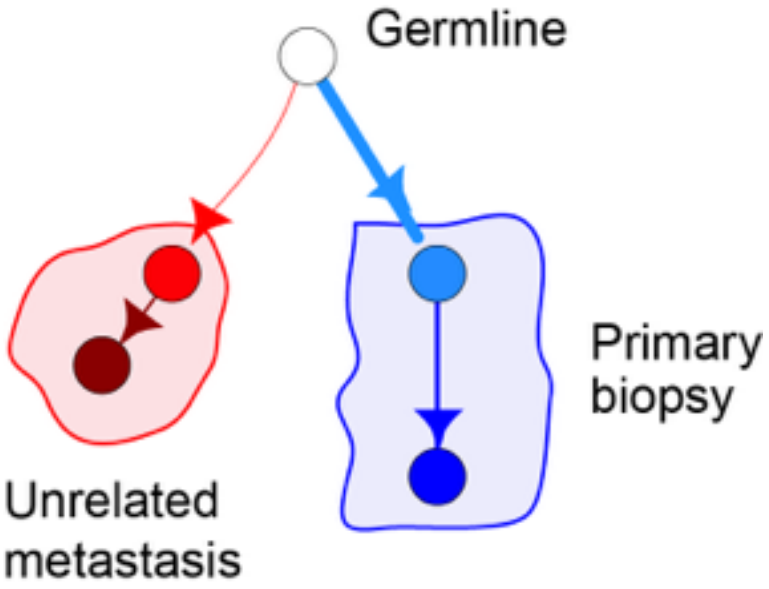


Inferred phylogeny and tissue sampling

Unrelated primary and metastasis samples

4 brain metastasis/primary cases



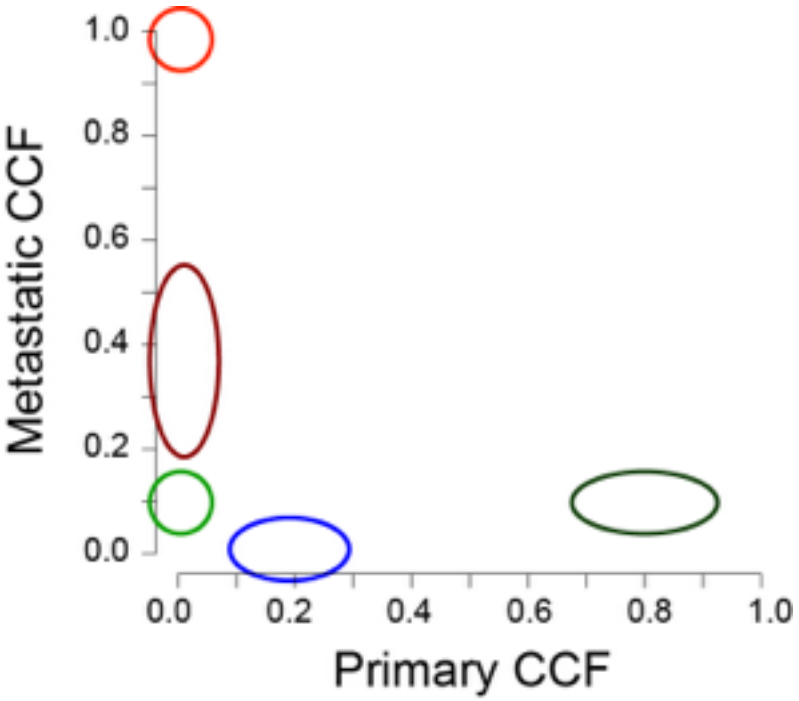


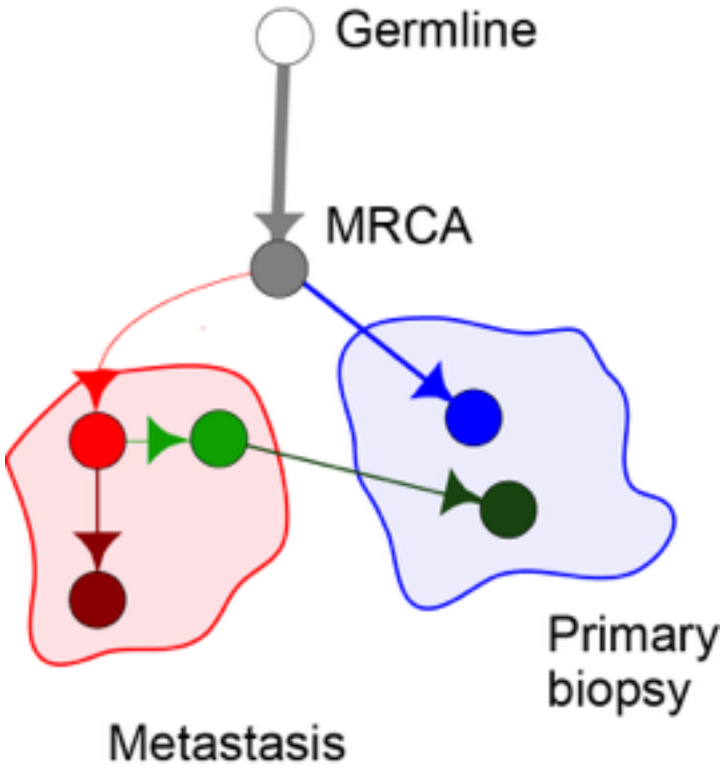
Modeled mutation data

Inferred phylogeny and tissue sampling

Primary tumor self-seeding by the metastasis

0 brain metastasis/primary cases



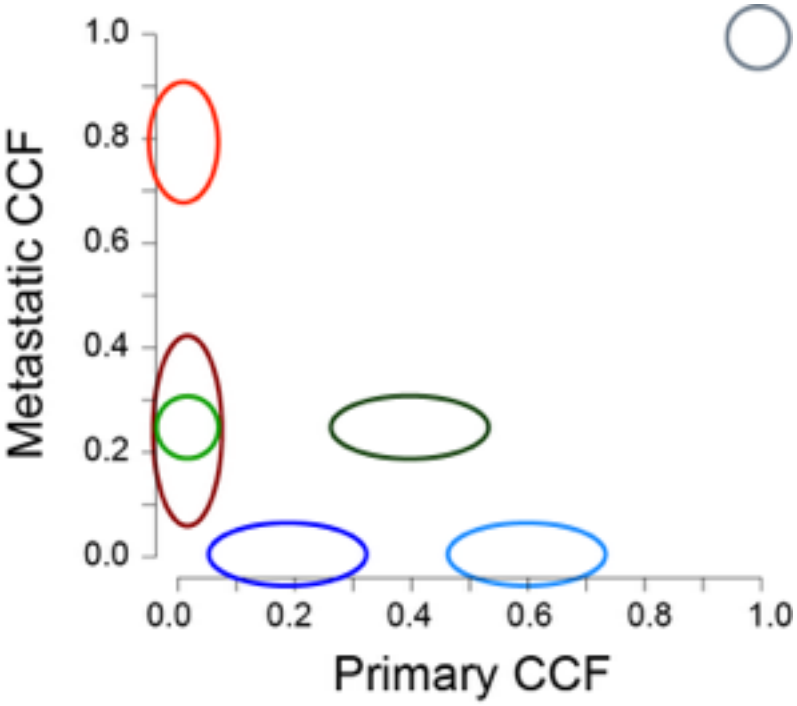


Modeled mutation data

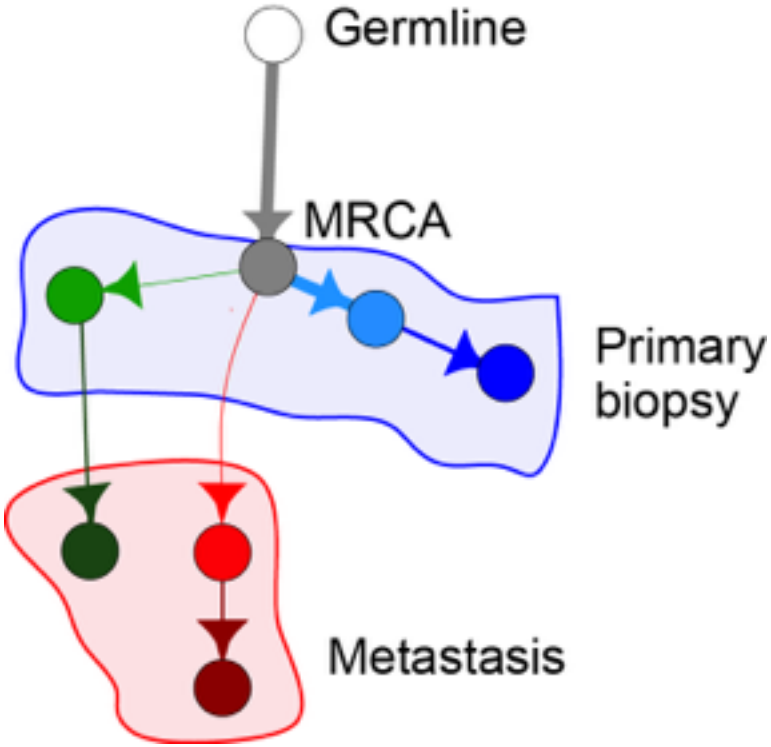
Inferred phylogeny and tissue sampling

Polyclonal seeding of metastasis

0 brain metastasis/primary cases



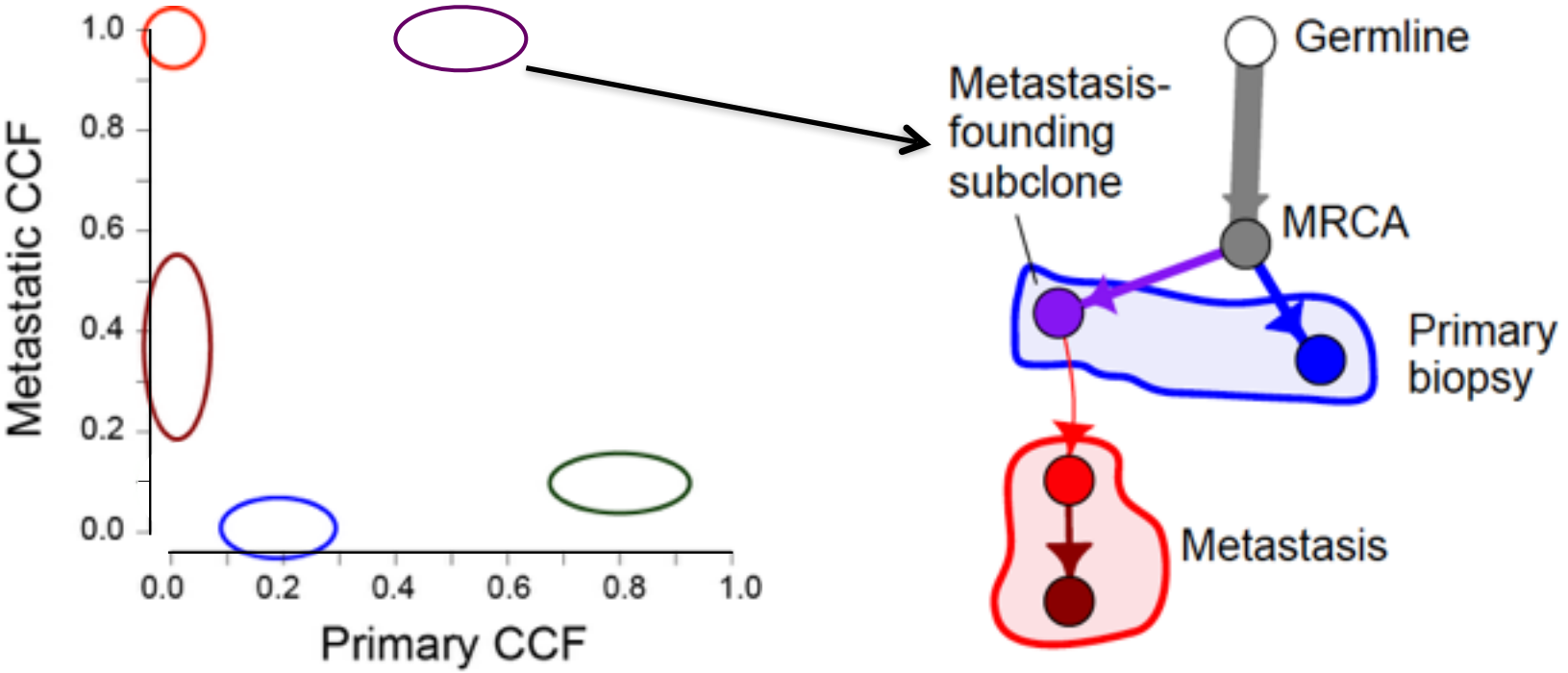
Modeled mutation data



Inferred phylogeny and tissue sampling

Metastasis-founding subclone

0 brain metastasis/primary cases



Modeled mutation data

Inferred phylogeny and tissue sampling

Question: Do brain metastases harbor clinically actionable mutations absent in their primary tumors?

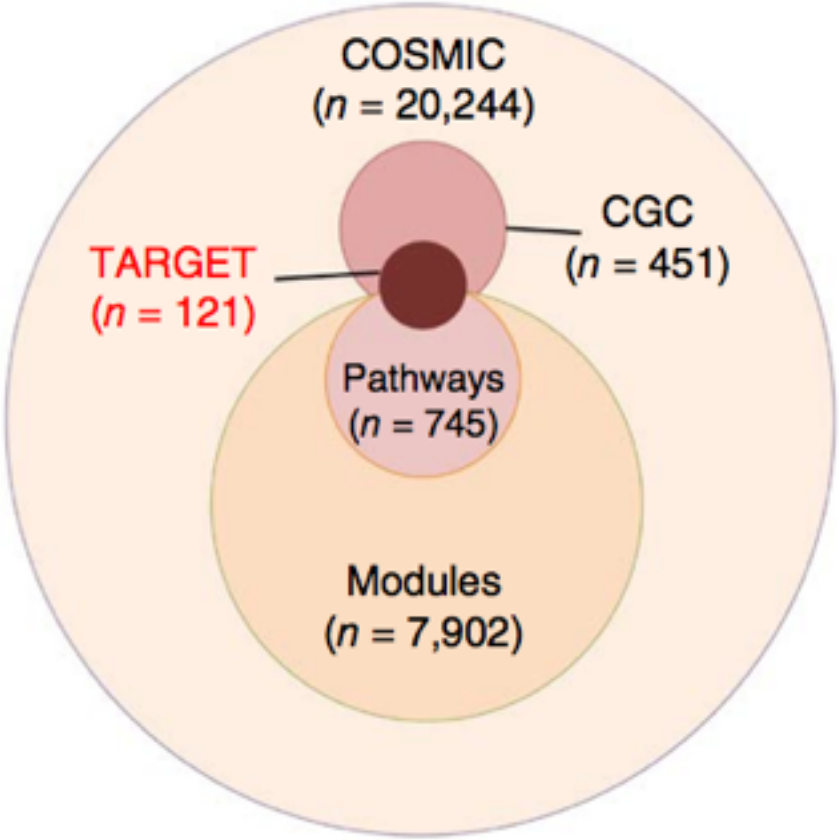
Definition of ‘clinically actionable’ mutations

TARGET database: 121 genes with criteria for nomination as ‘actionable’:

- Tumor suppressors: biallelic inactivation
- Oncogenes: amplification, hotspot mutation

Genes/mutations connected with specific treatment opportunities

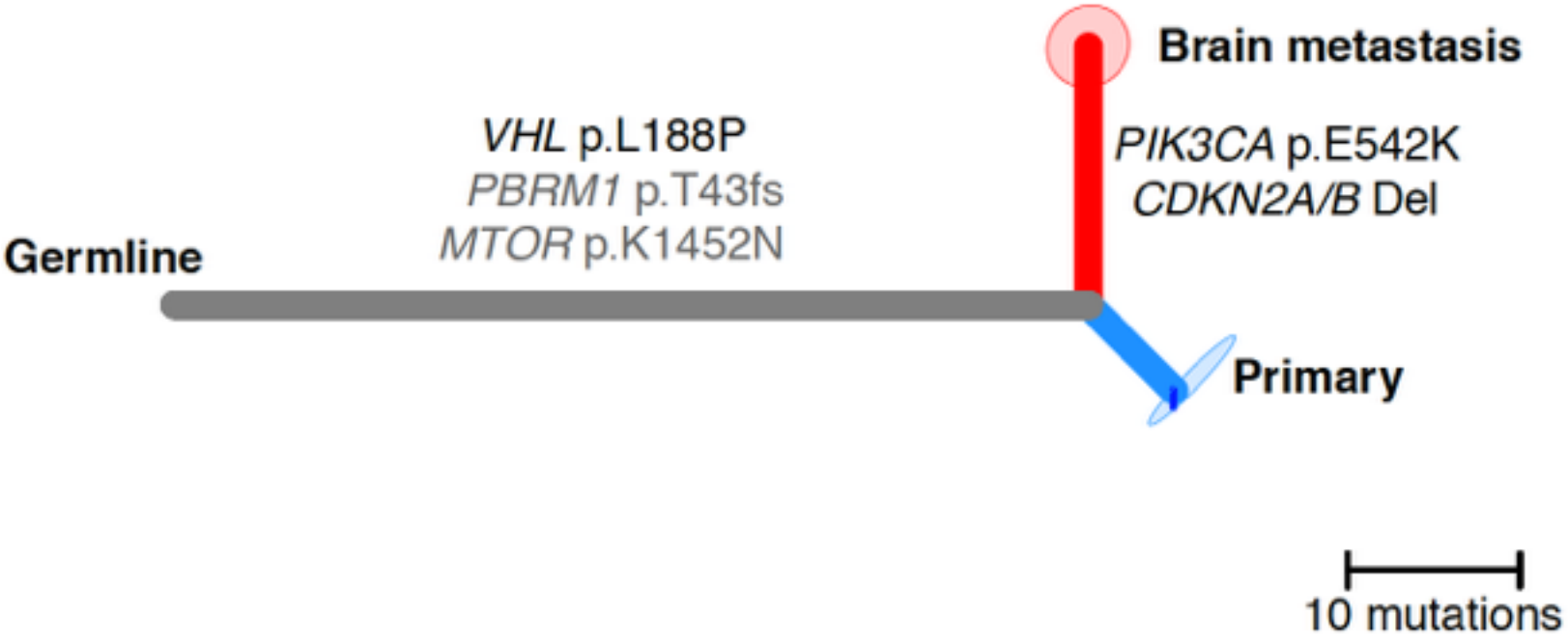
Many in the context of a **genomically guided clinical trial**



Van Allen et al., *Nature Medicine* 2014

Brain metastases are genetically distinct from clinically sampled primary tumors

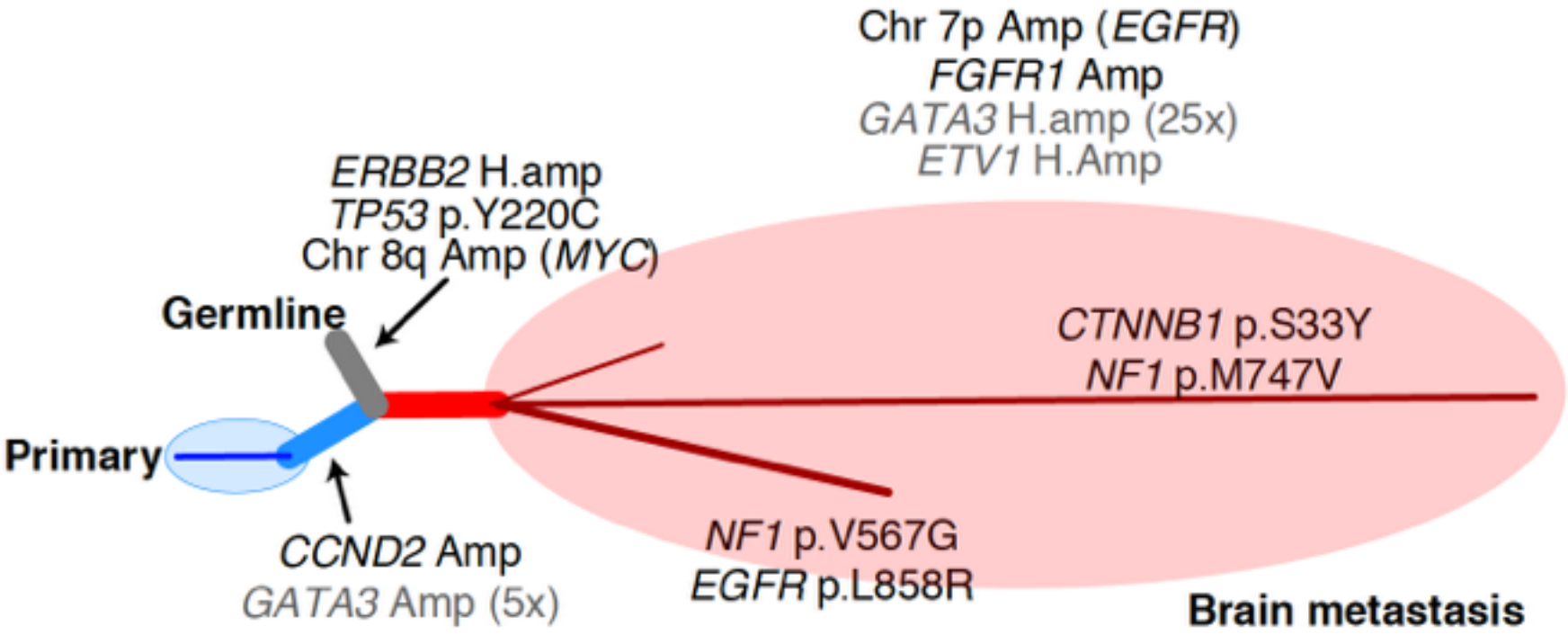
Renal cell carcinoma



Brastianos, Carter et al., *Cancer Discovery 2015*

Example: subclonal clinically actionable mutation in *EGFR* not detected in primary

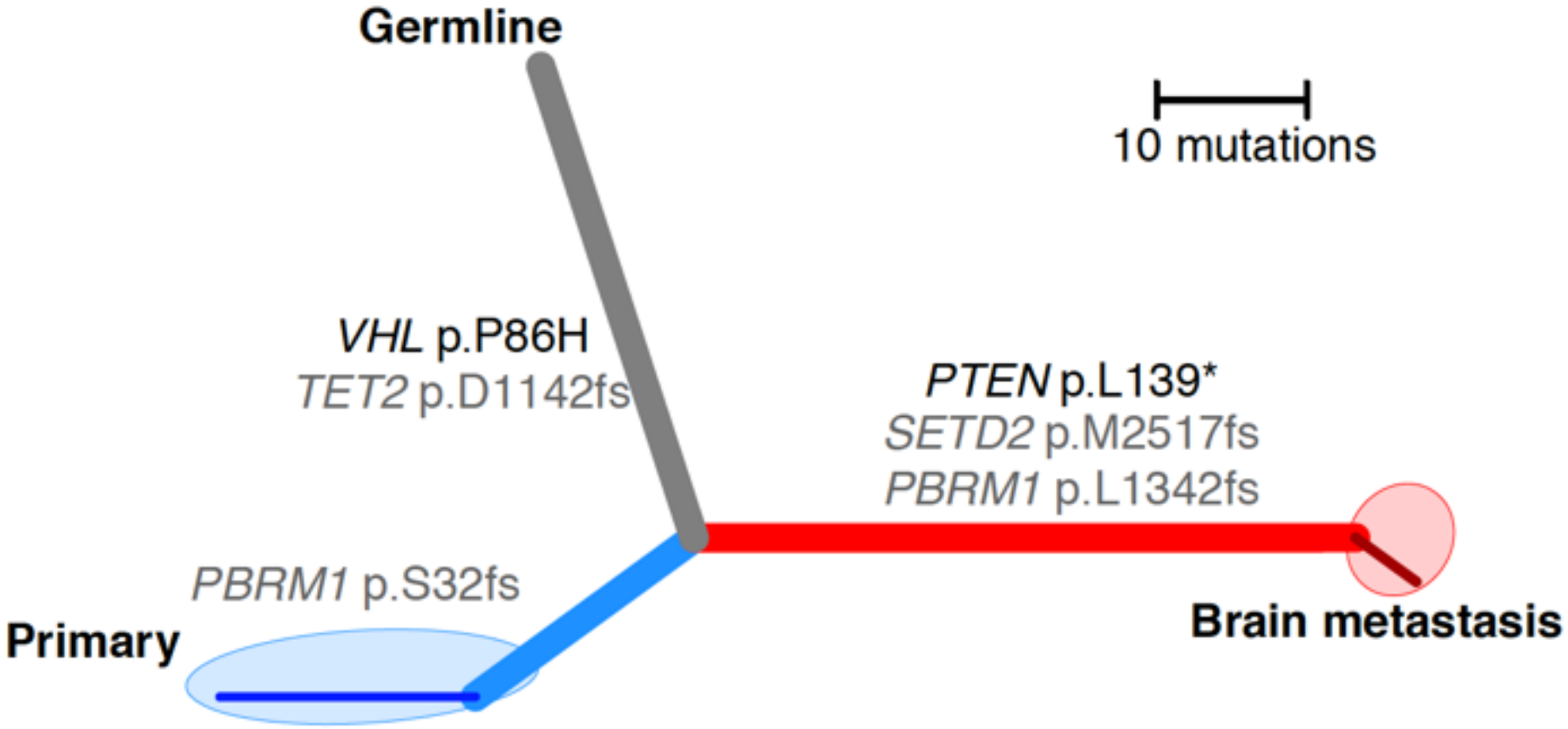
HER2+ breast carcinoma



Brastianos, Carter et al., *Cancer Discovery 2015*

Example: clinically actionable *PTEN* loss not detected in primary

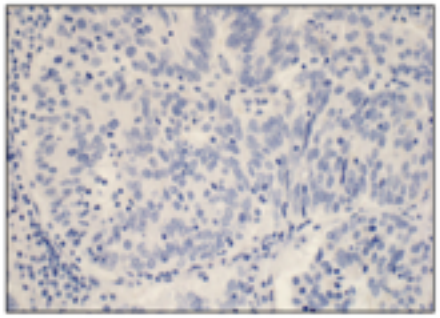
Renal cell carcinoma



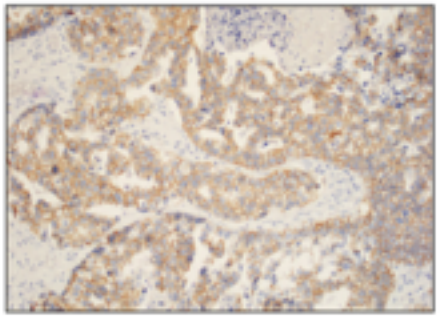
Example: subclonal clinically actionable mutation in *ERBB2* not present in primary

Ovarian carcinoma

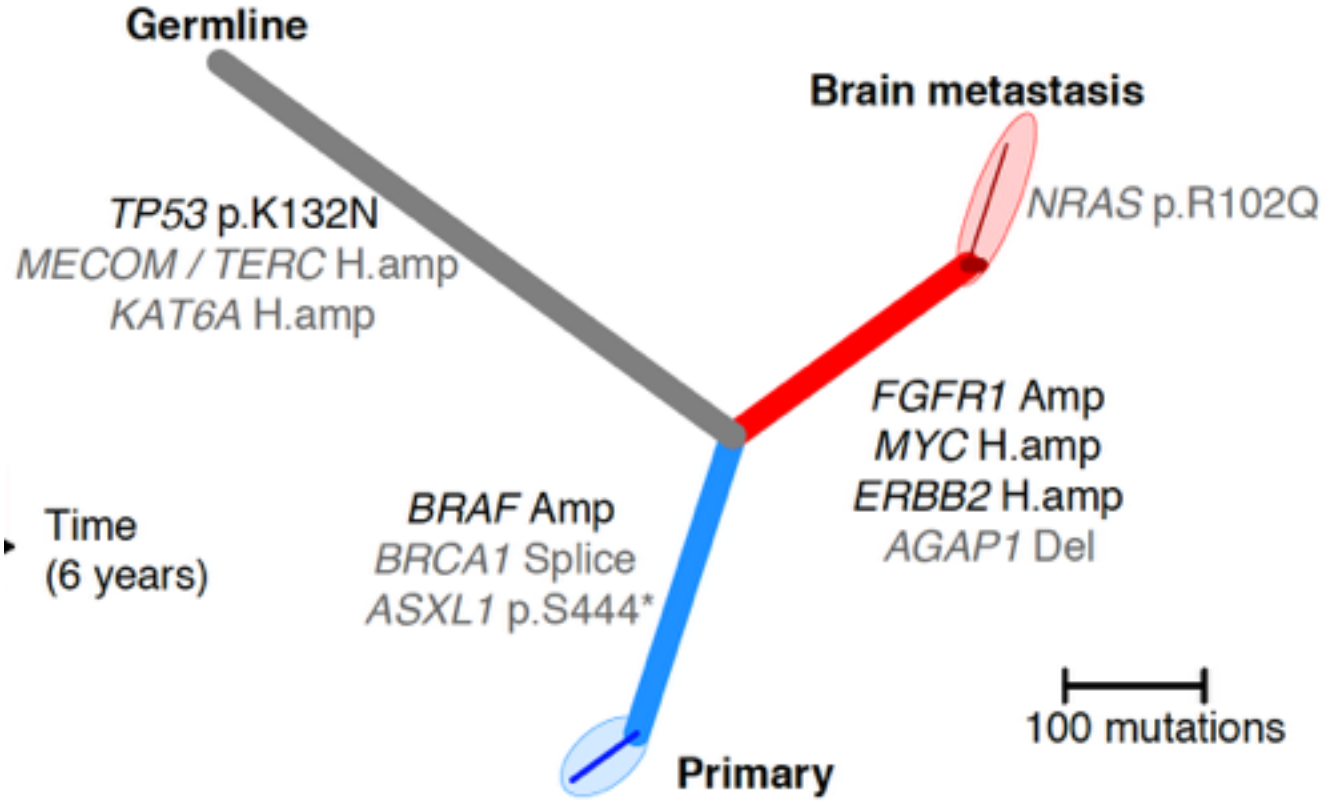
HER2 IHC



Primary tumor

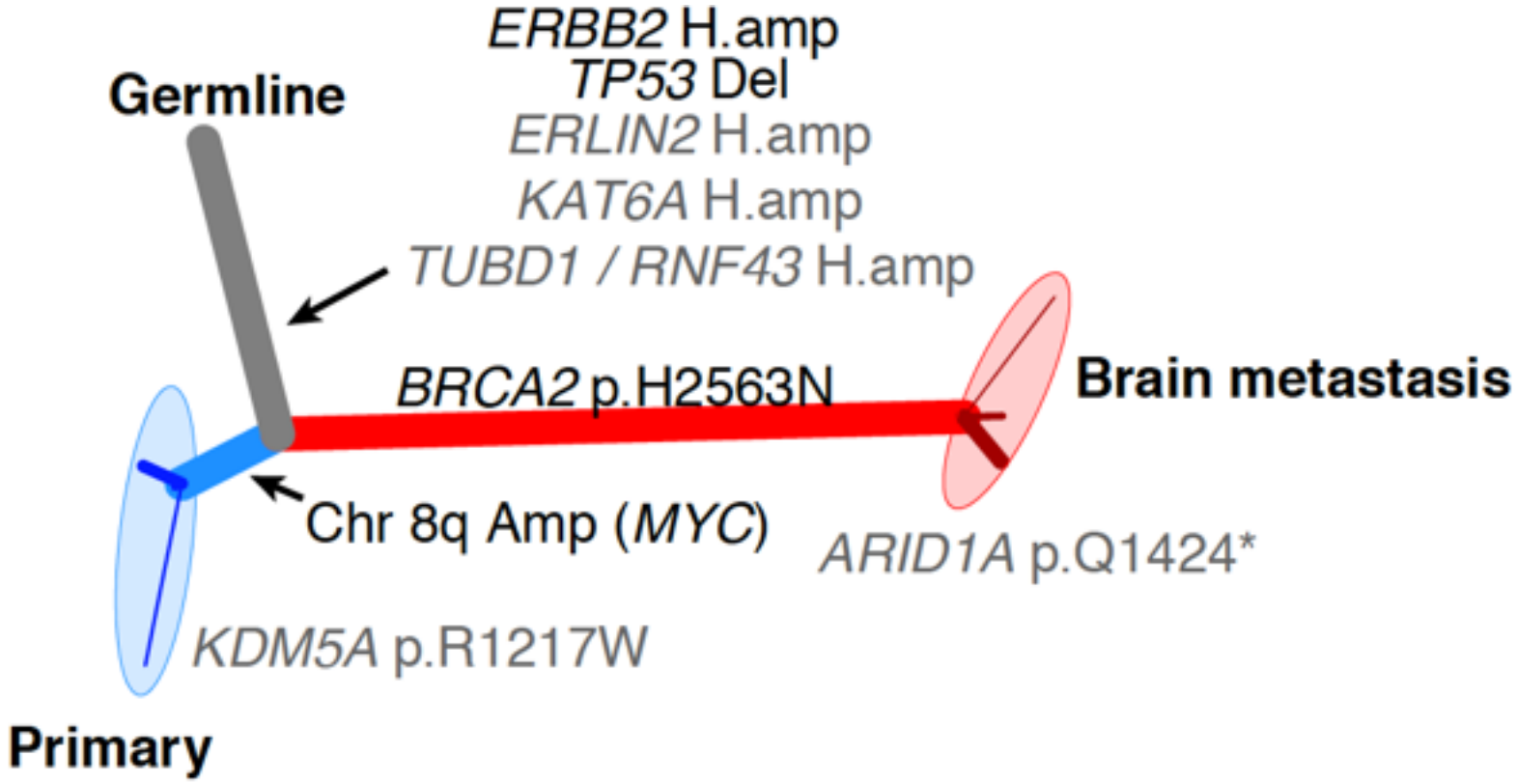


Brain metastasis

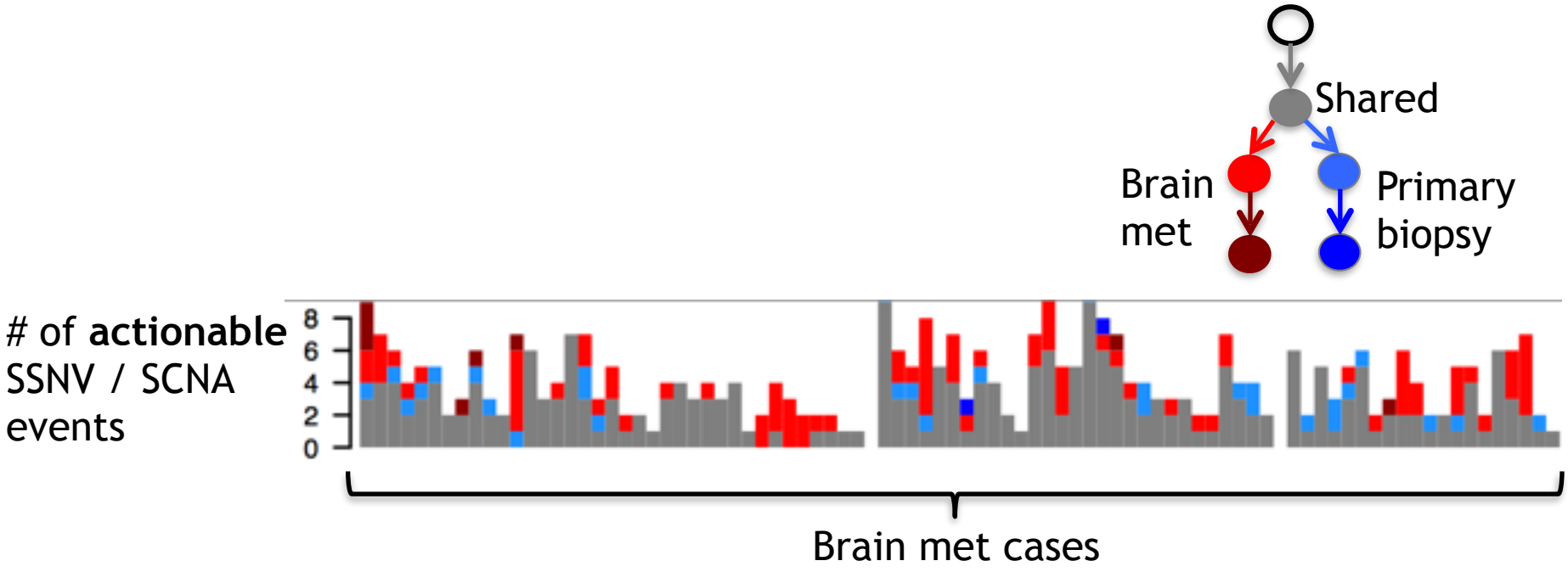


Brain metastases are genetically distinct from clinically sampled primary tumors

HER2+ breast carcinoma

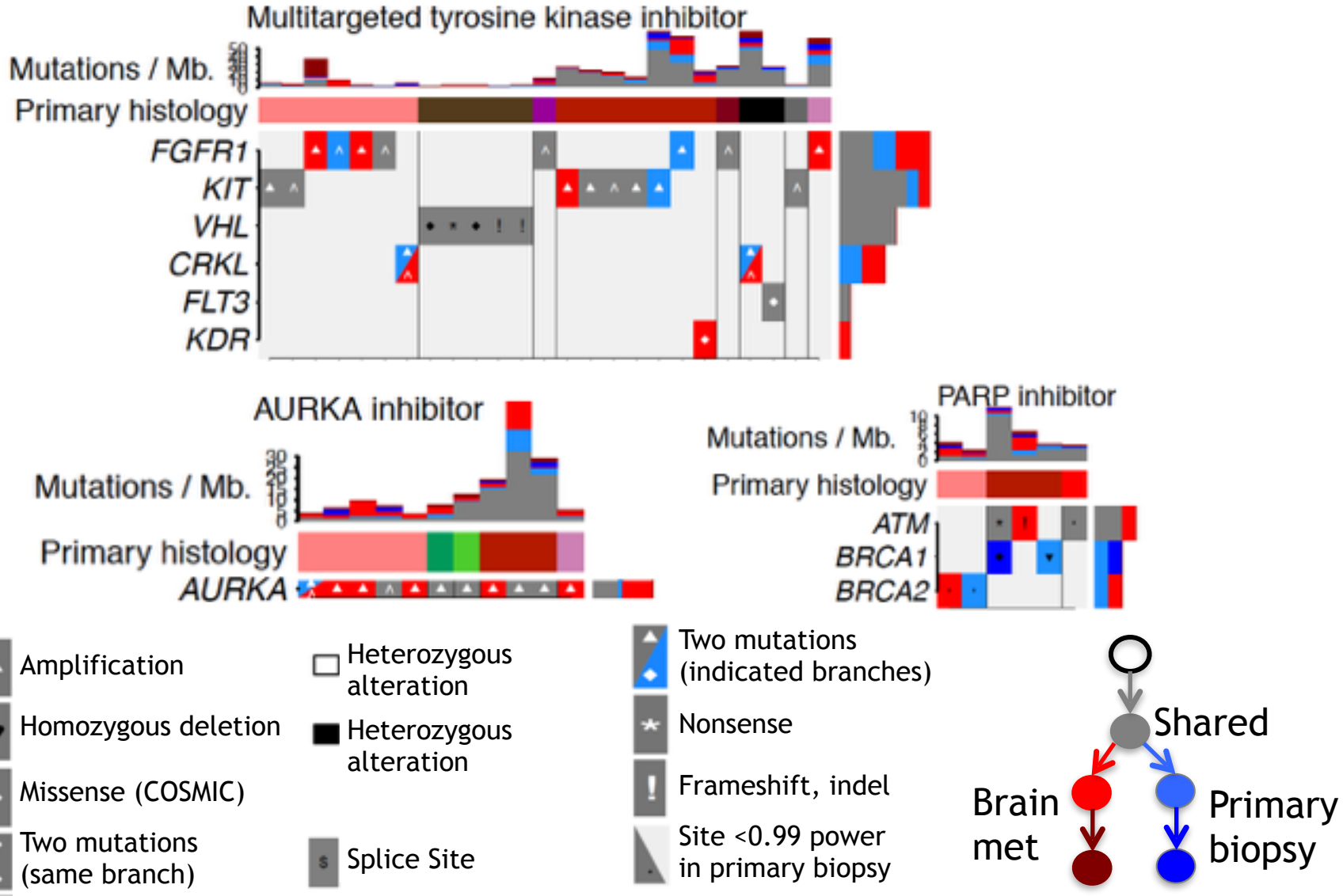


Clinically actionable alterations occur in all phylogenetic branches

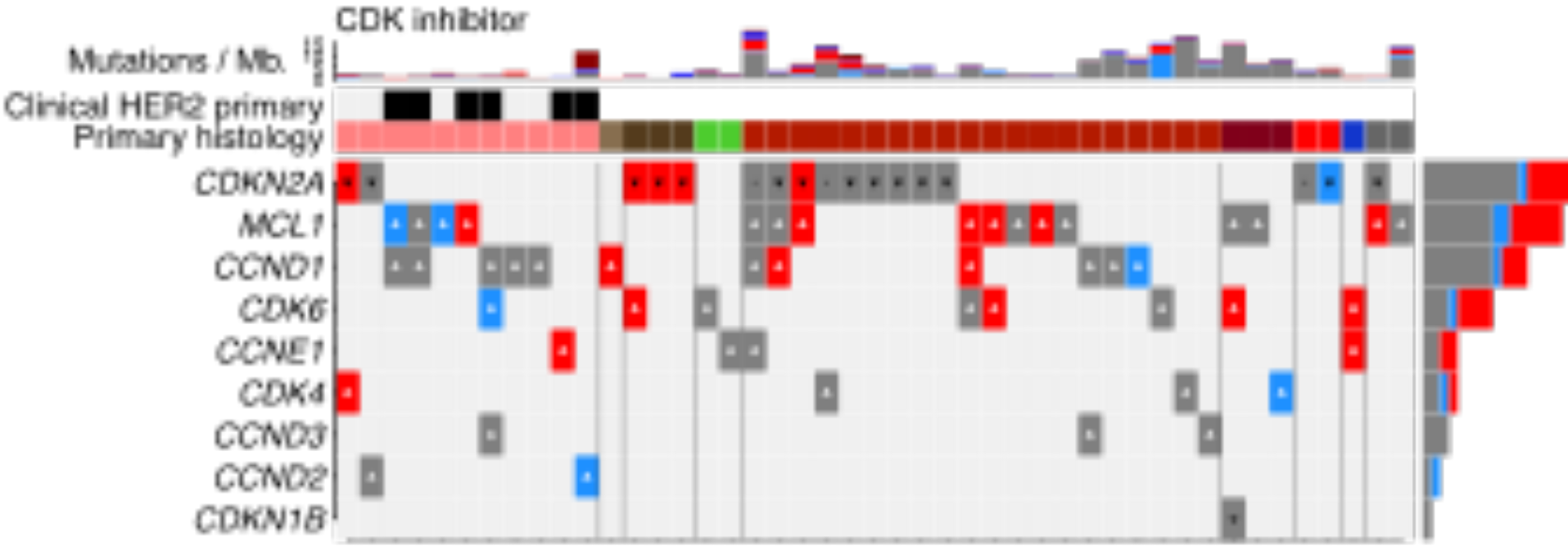


53% of cases have a clinically actionable alteration in the brain metastasis, not detected in the primary biopsy.

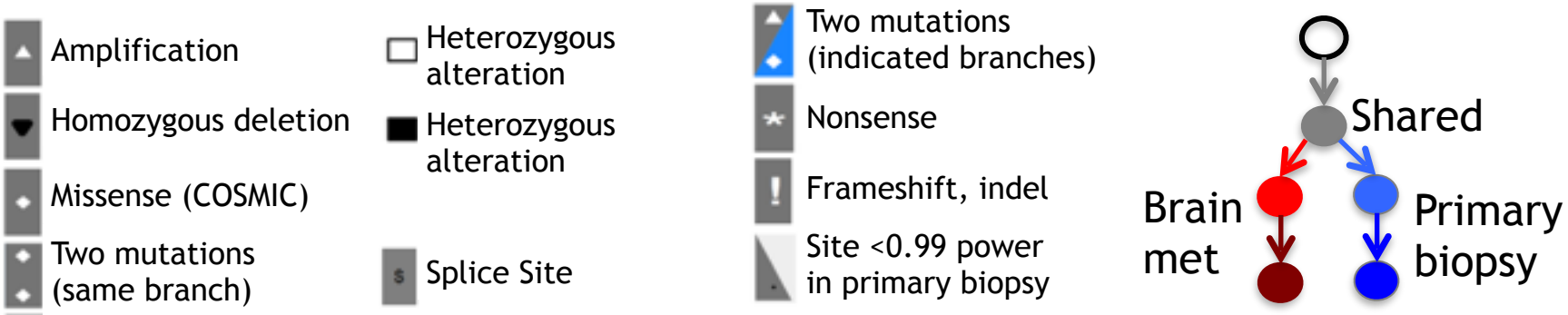
Clinically actionable alterations occur in all phylogenetic branches



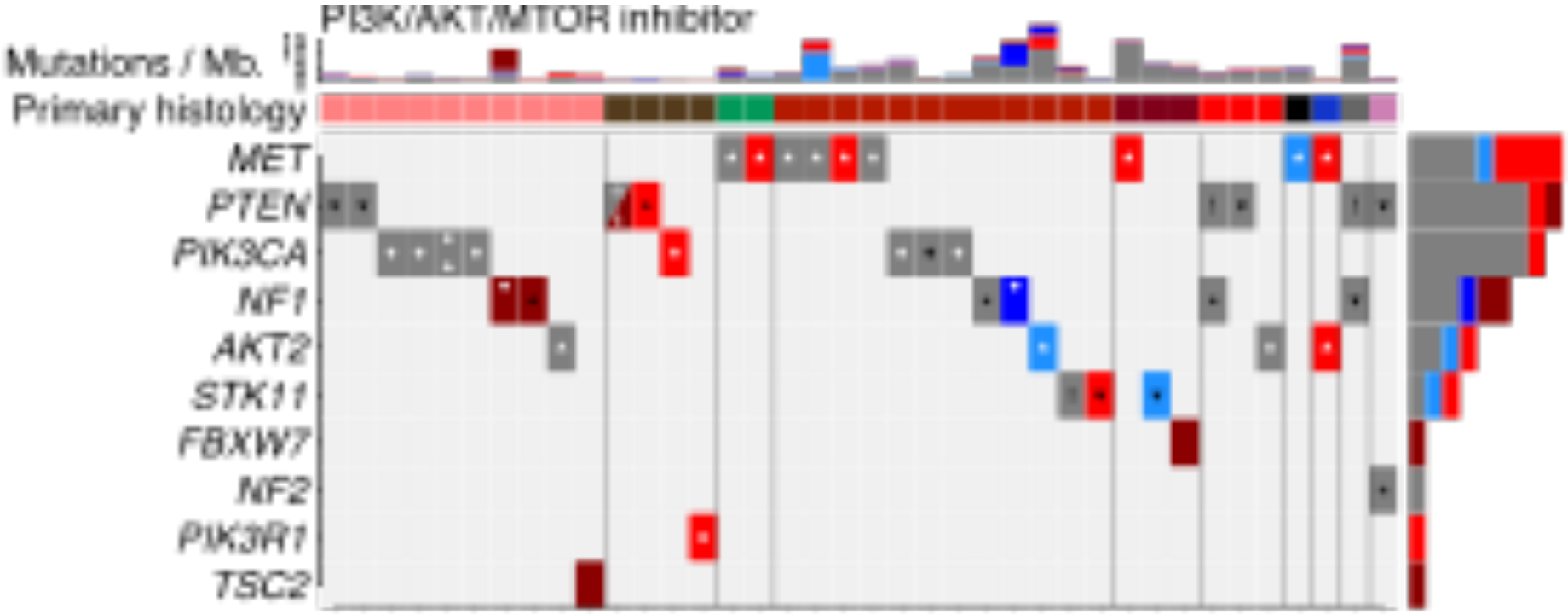
Opportunities to target brain metastases



51% of cases with alterations predicting sensitivity to CDK inhibitor

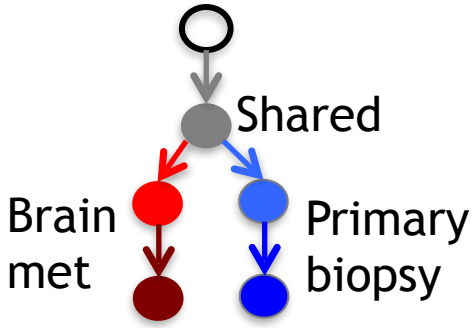


Opportunities to target brain metastases

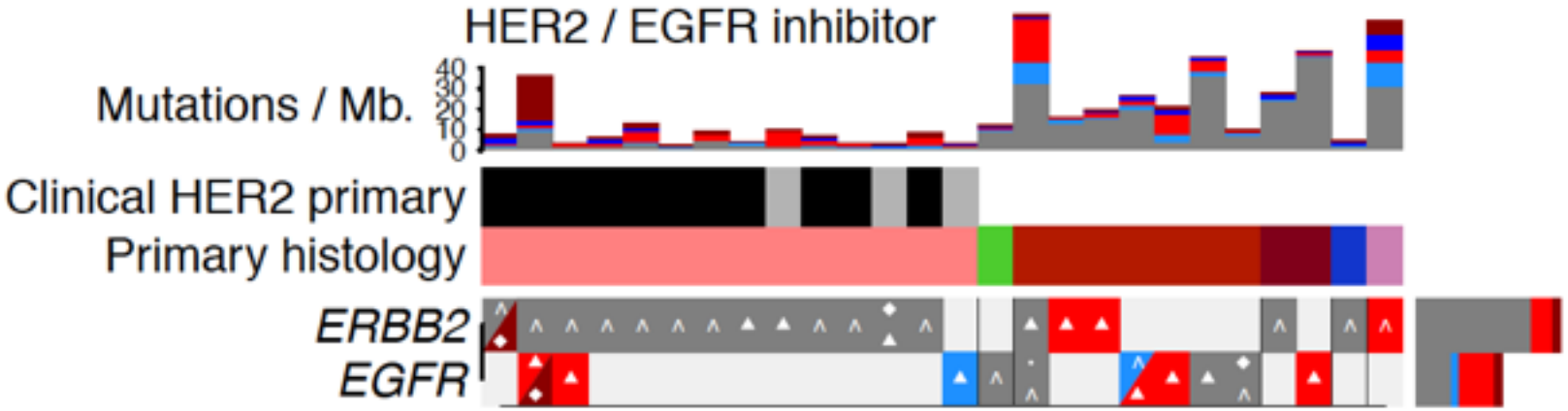


43% of cases with alterations predicting sensitivity to PI3K/AKT/mTOR inhibitor

- Amplification
- Homozygous deletion
- Missense (COSMIC)
- Two mutations (same branch)
- Heterozygous alteration
- Heterozygous alteration
- Splice Site
- Two mutations (indicated branches)
- Nonsense
- Frameshift, indel
- Site <0.99 power in primary biopsy



HER2/EGFR Alterations



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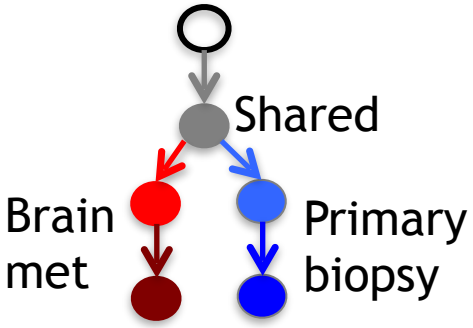
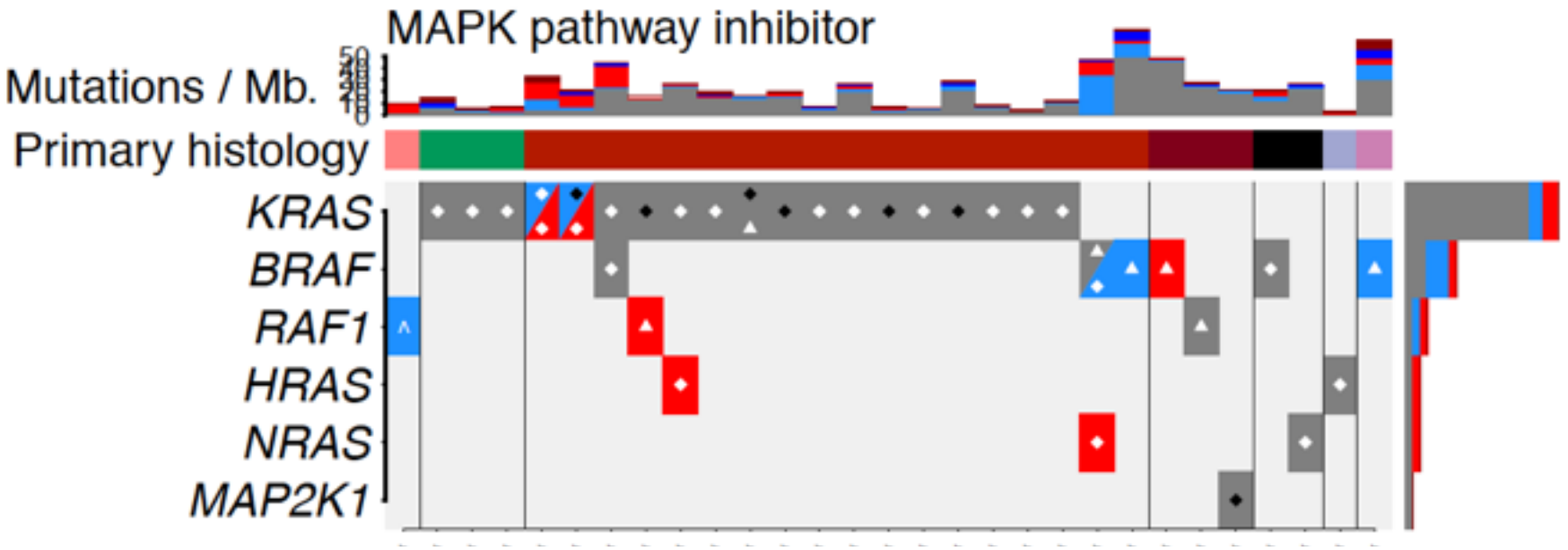


MAPK pathway



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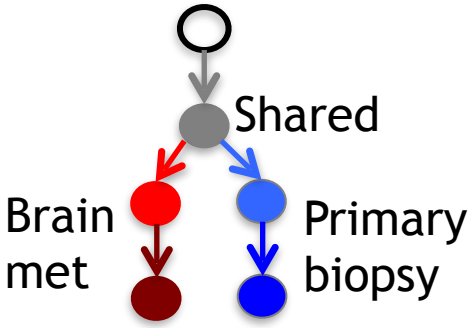


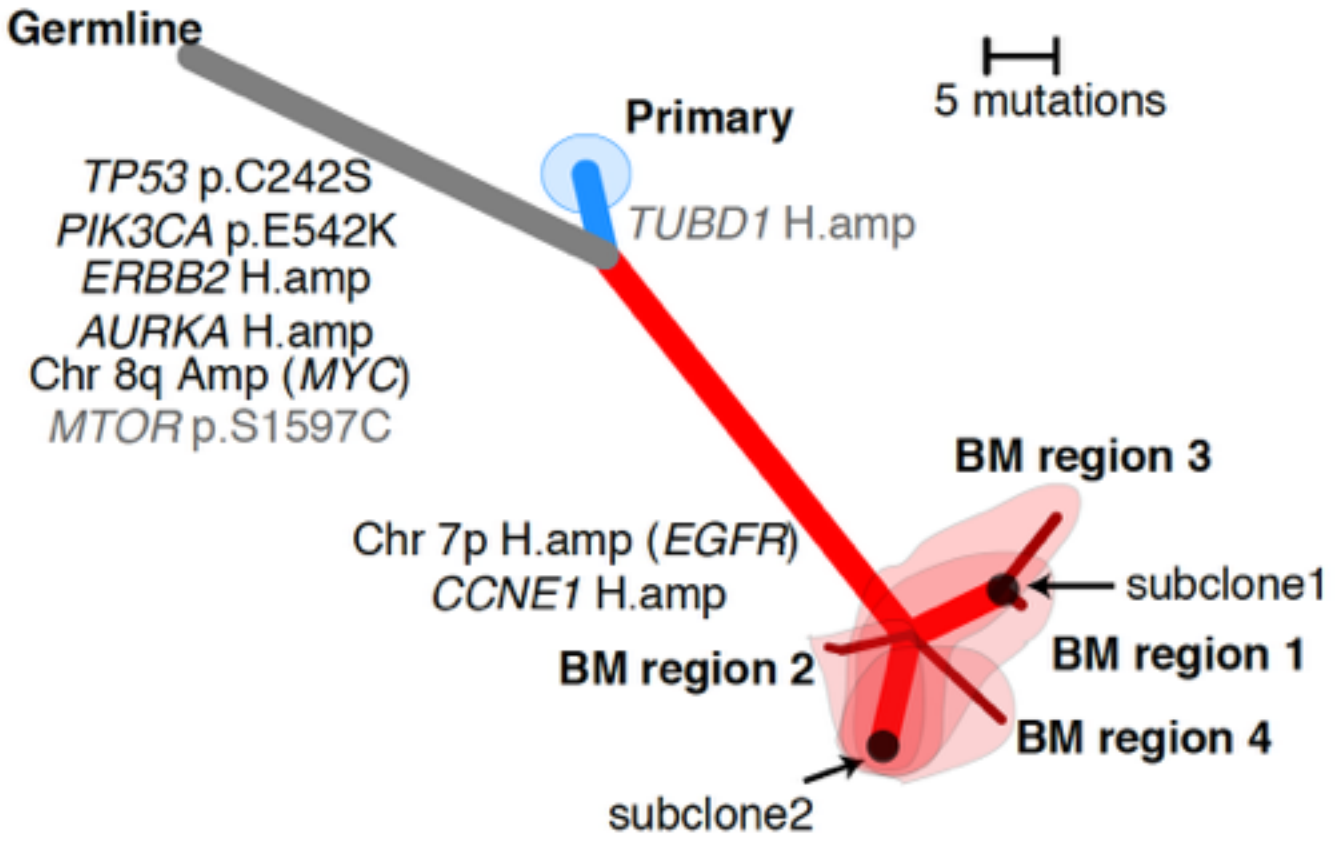
Question: what about regional heterogeneity within the brain?

- Genetic divergence between primary and metastatic samples poses a major challenge to clinical decision making

- How representative of bulk CNS metastatic disease is a single brain metastasis sample?

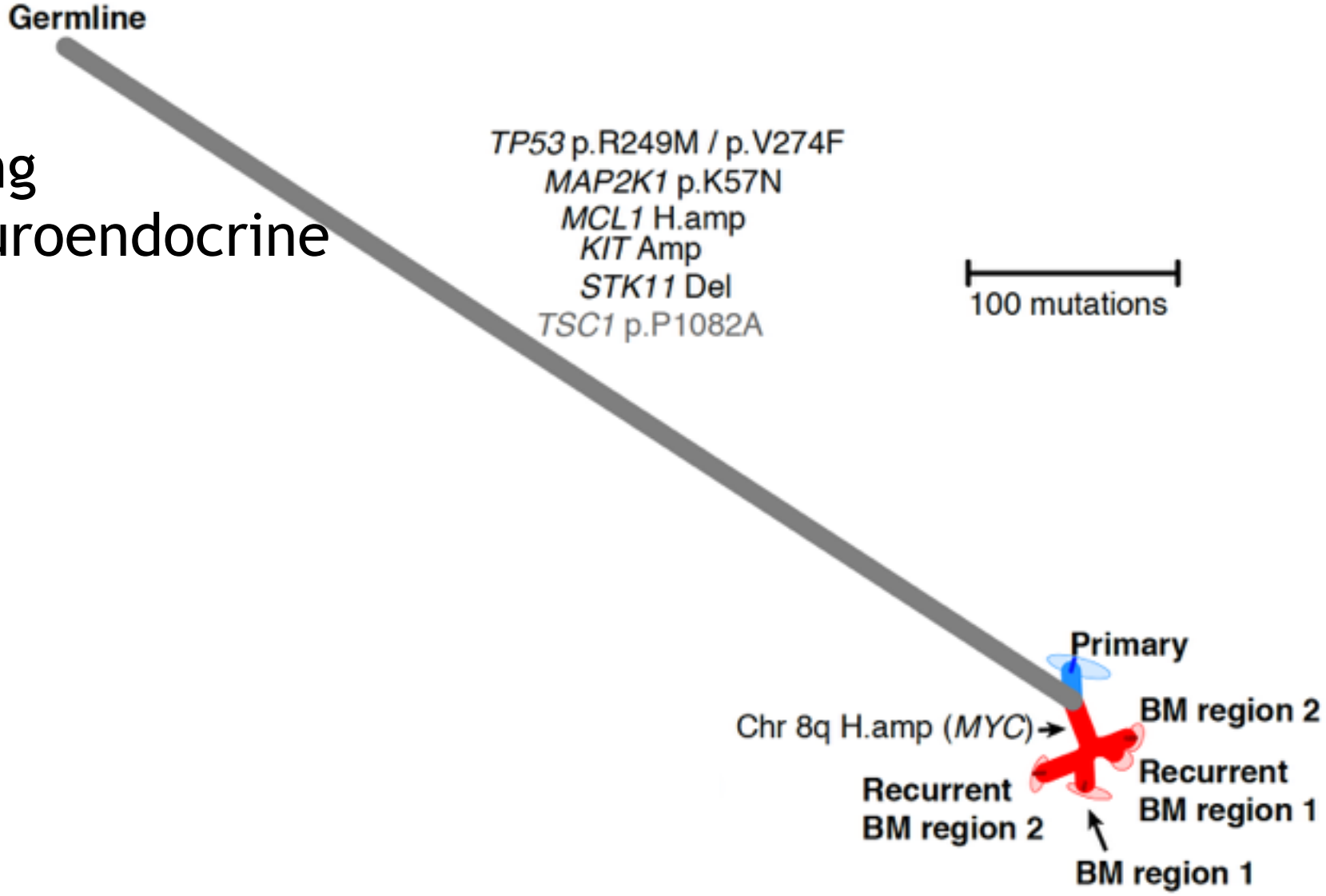
Four regions of a solitary brain metastasis are homogeneous

HER2+ breast cancer



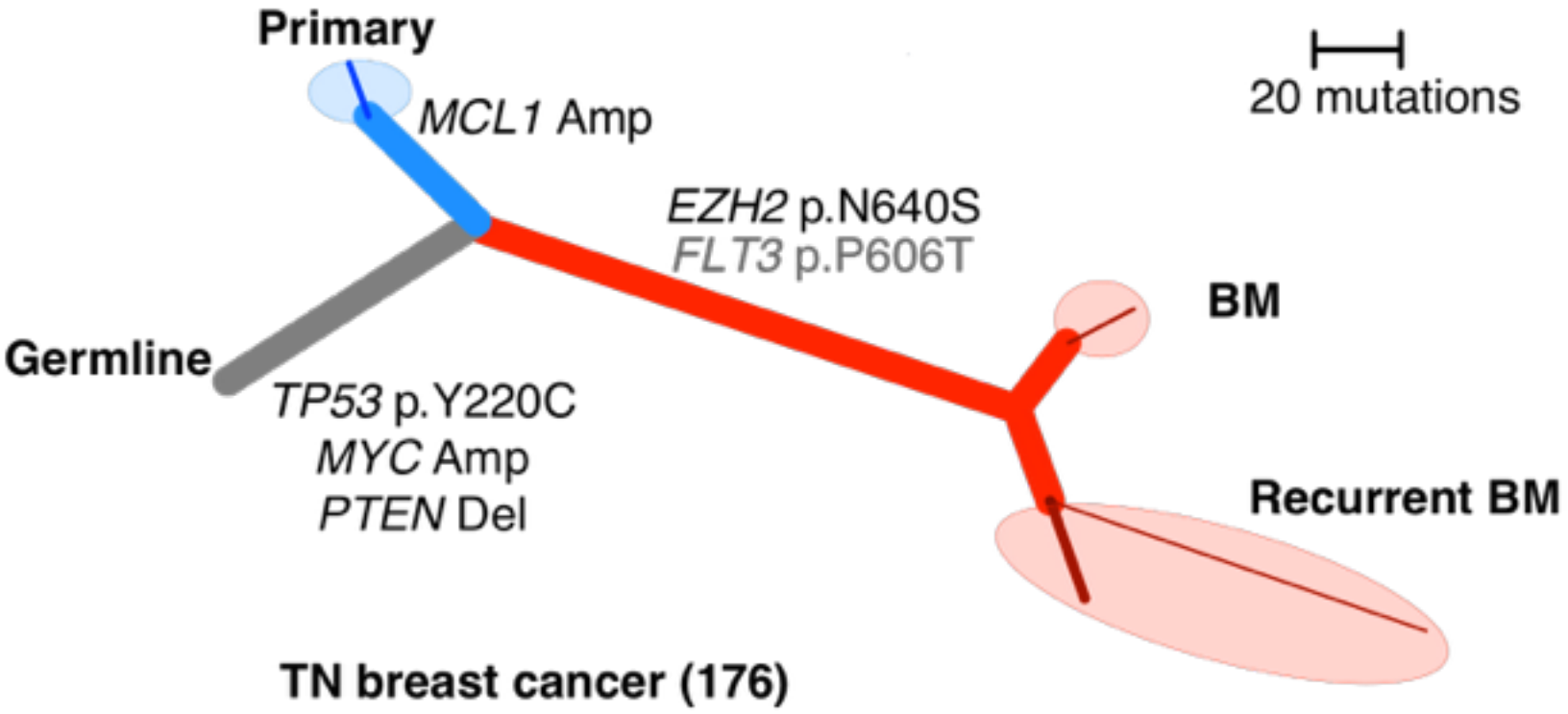
Temporally distinct brain metastases share all actionable drivers

Lung neuroendocrine



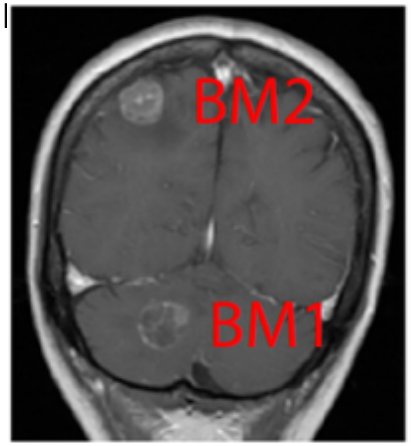
Temporally distinct brain metastases are homogeneous

Triple Negative Breast Cancer

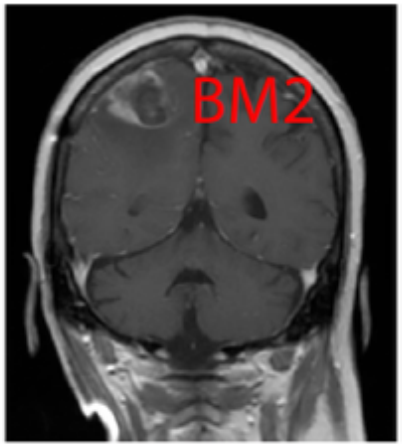


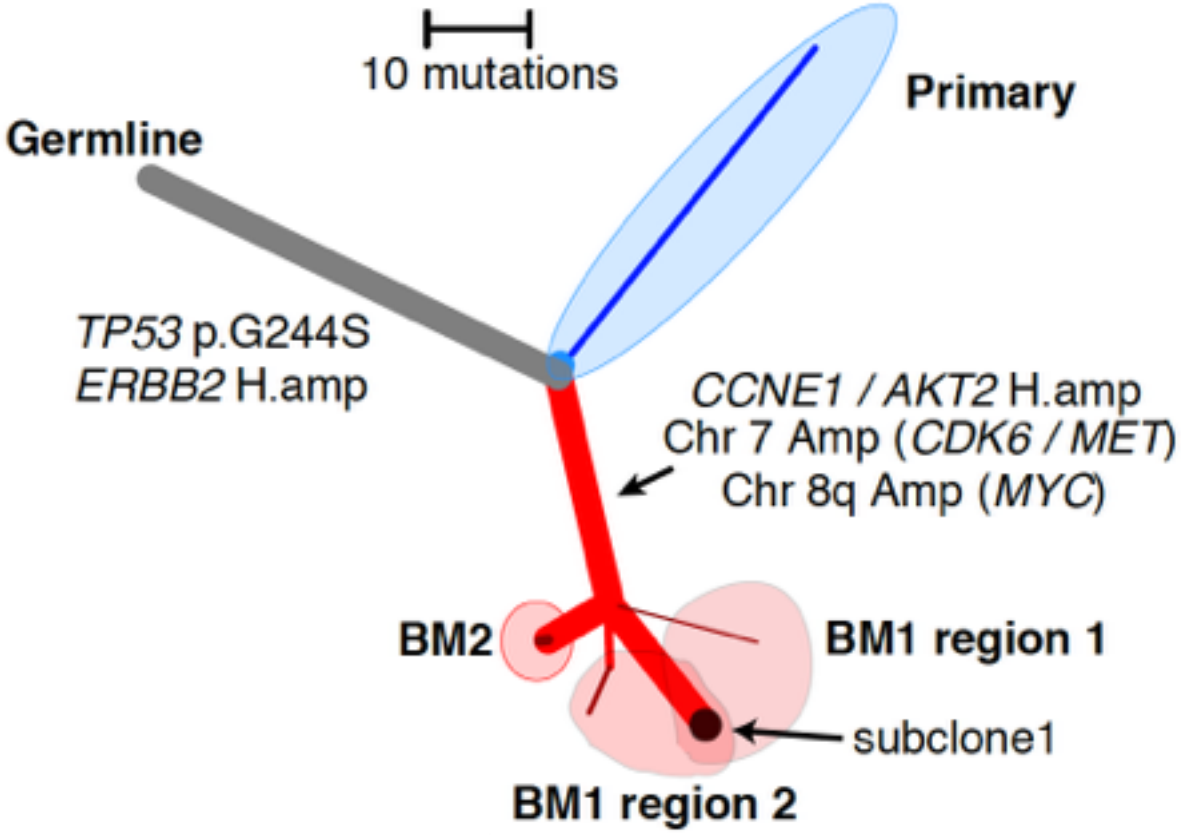
Anatomically distinct brain metastases share all actionable drivers

Pre-XRT, pre-resection cerebellar



Post-XRT, pre-resectior of parietal met

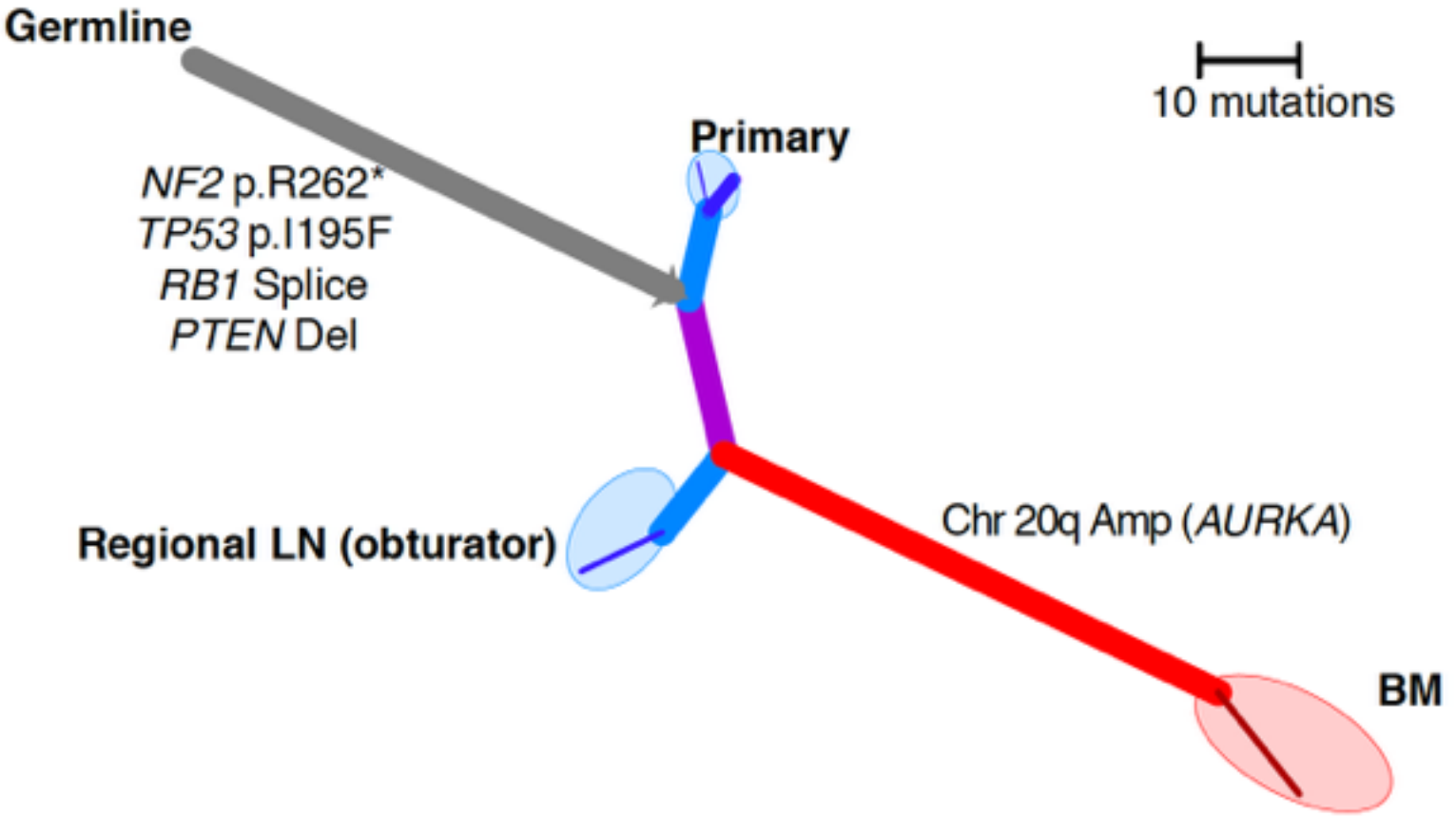




Question: To what extent do extracranial sites recapitulate genetic vulnerabilities in brain metastases?

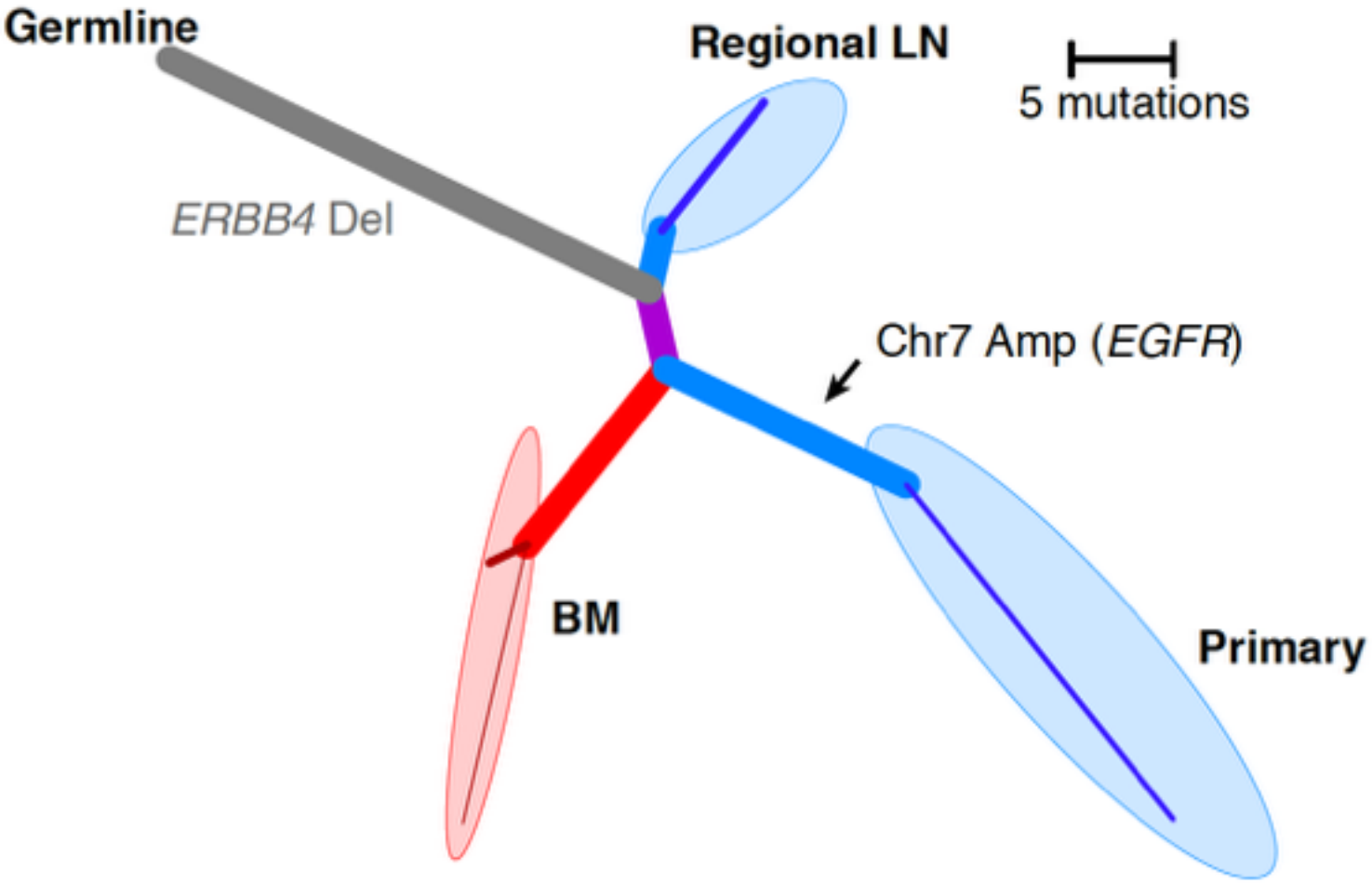
Example: Lymph node not reliable genetic surrogate of brain metastasis

Serous ovarian cancer



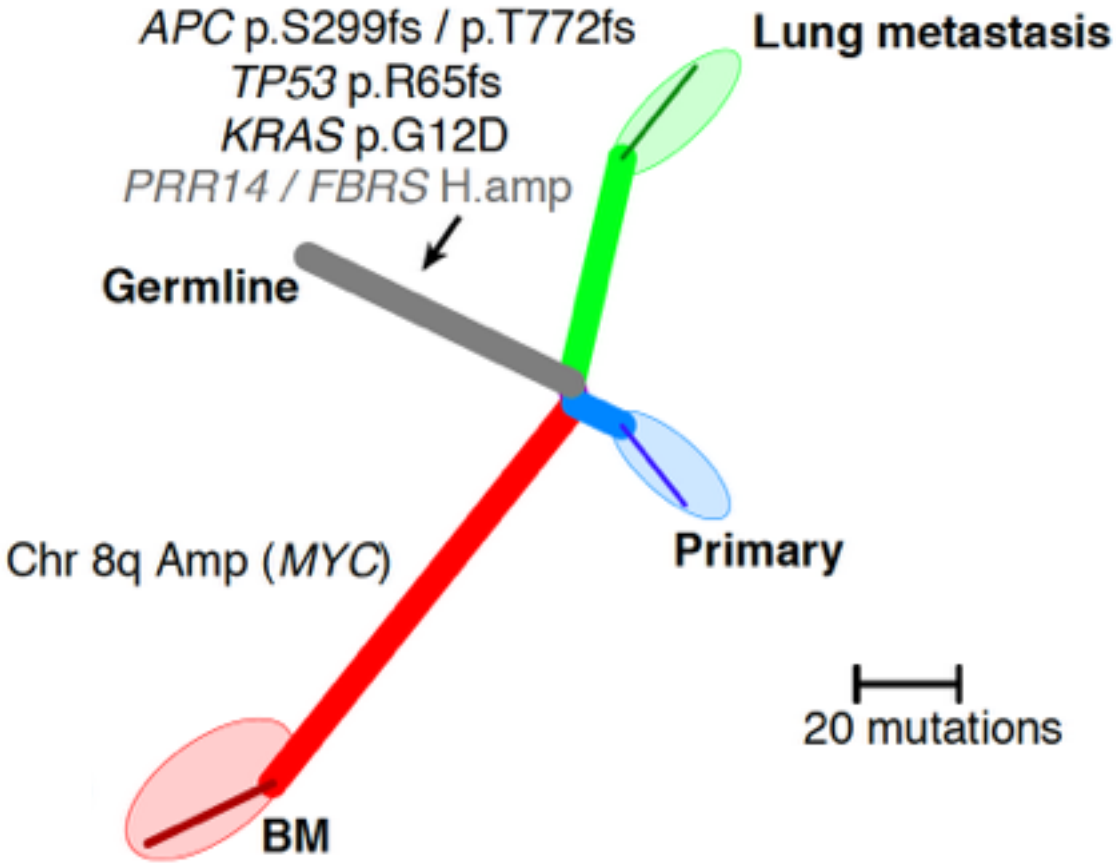
Example: Extracranial sites not reliable genetic surrogate of brain metastasis

Triple negative breast cancer



Example: Distal metastasis not reliable genetic surrogate of brain metastases

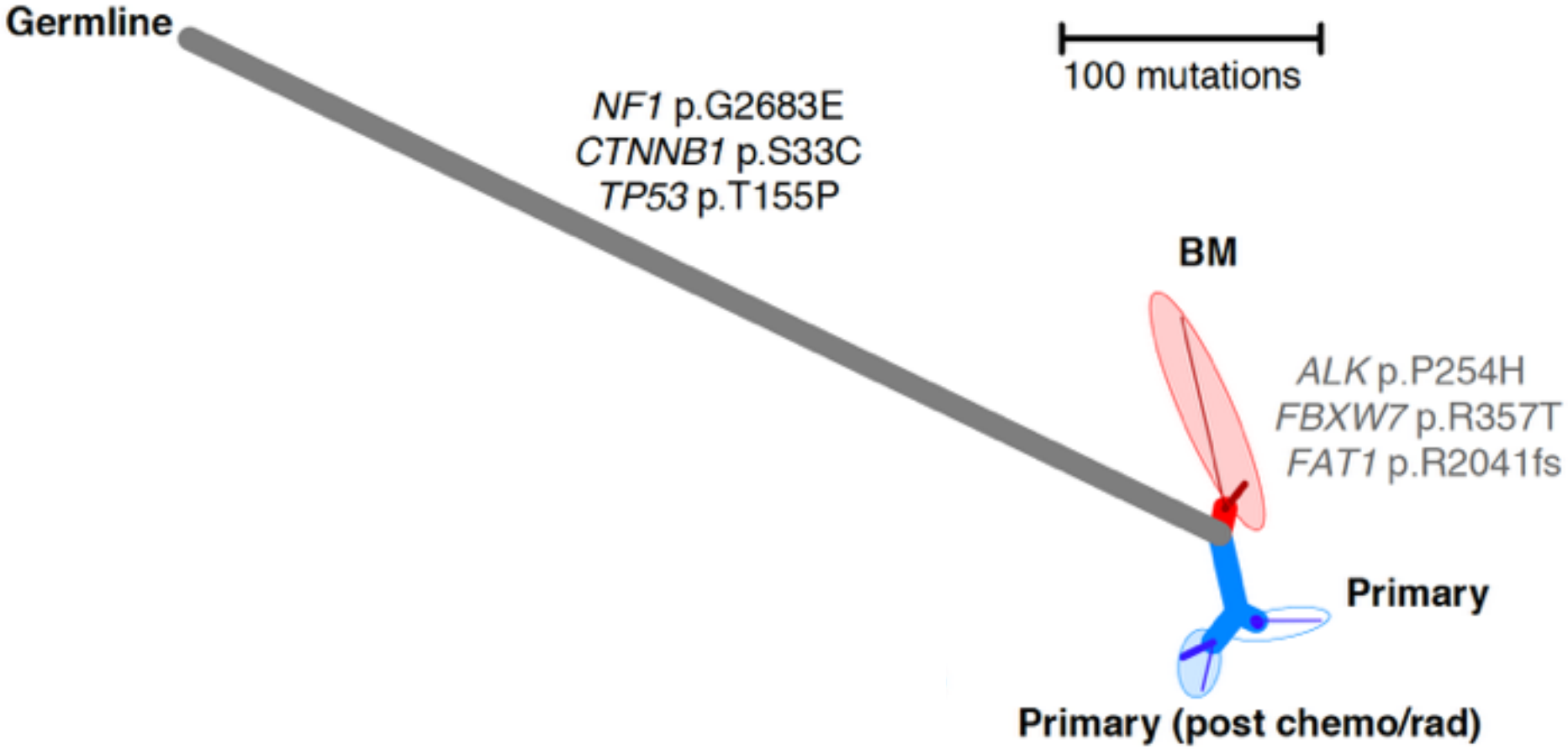
Colorectal carcinoma



Brastianos, Carter et al., *Cancer Discovery 2015*

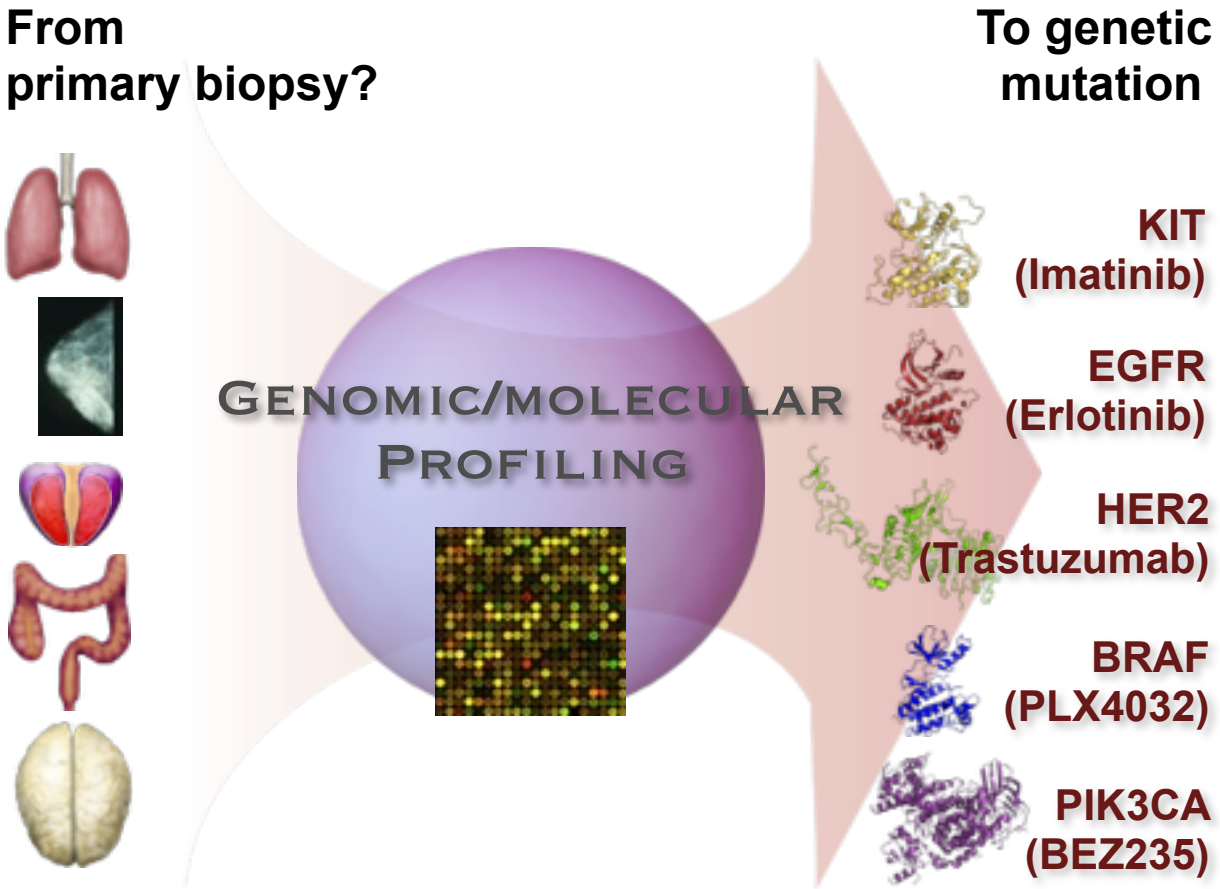
Pre/post treatment primary regions more related to each other than to brain met

Primary lung carcinoma



Brain metastases are genetically divergent from primary tumors and other extracranial sites.

Important opportunities for precision medicine



53% of cases had at least one clinically actionable genetic alteration in the brain metastasis, not detected in the primary tumor.

Conclusions

- Every metastasis displayed branched evolution.
- Brain metastases harbored distinct clinically actionable genetic alterations, compared to their primary tumors.
- All brain metastasis regions harbored the same actionable alterations.
- Extracranial metastases are not a reliable surrogate for brain metastases.

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