Lessons about tumour biology from ctDNA at diagnosis & during treatment and surveillance

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Using ctDNA to help diagnose cancer

- Cancer tissue of origin defines the disease course – and the treatment
- Treatment success varies by tissue of origin, and often depends on specific somatic alterations
- Sometimes we cannot biopsy a tumour – primary cannot be found, or is inaccessible

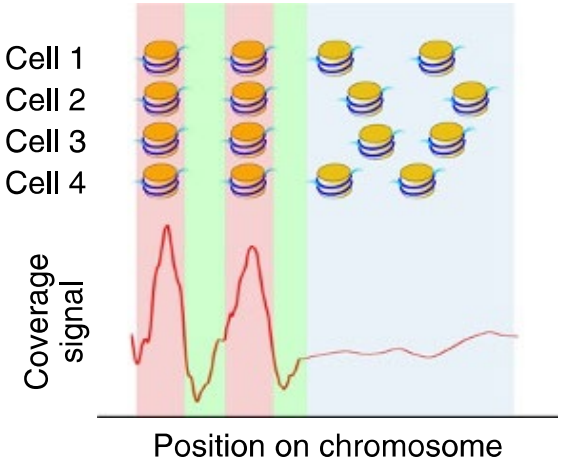
- **We can use ctDNA to diagnose tissue of origin and evaluate targetable mutations**

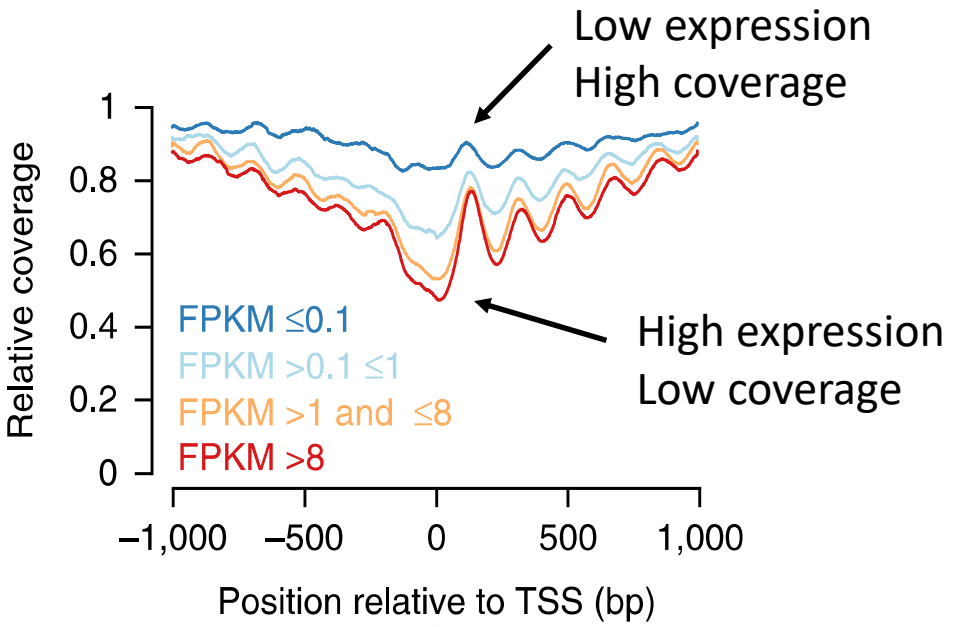
ctDNA fragments depends on nucleosome positions

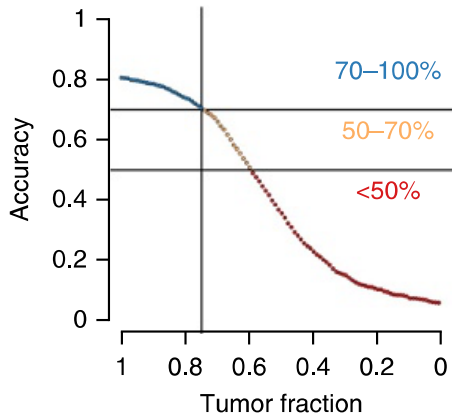
- So does gene expression!

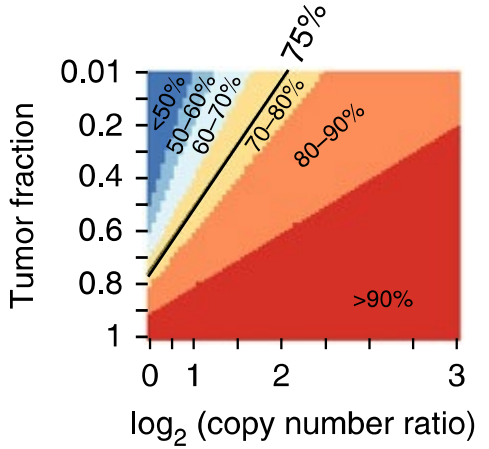
- Plasma from 104 healthy individuals show cfDNA mostly reflects hematopoietic cells
- Analysis of 426 plasma samples from cancer patients shows that in high tumour burden metastatic patients, transcription start site was identifiable from cancer driver genes with copy number amplification
- Requires high levels of ctDNA & amplification of target gene

Ulz et al, Nature Genetics 2016









ctDNA coverage can be used to infer high/low expression of specific genes

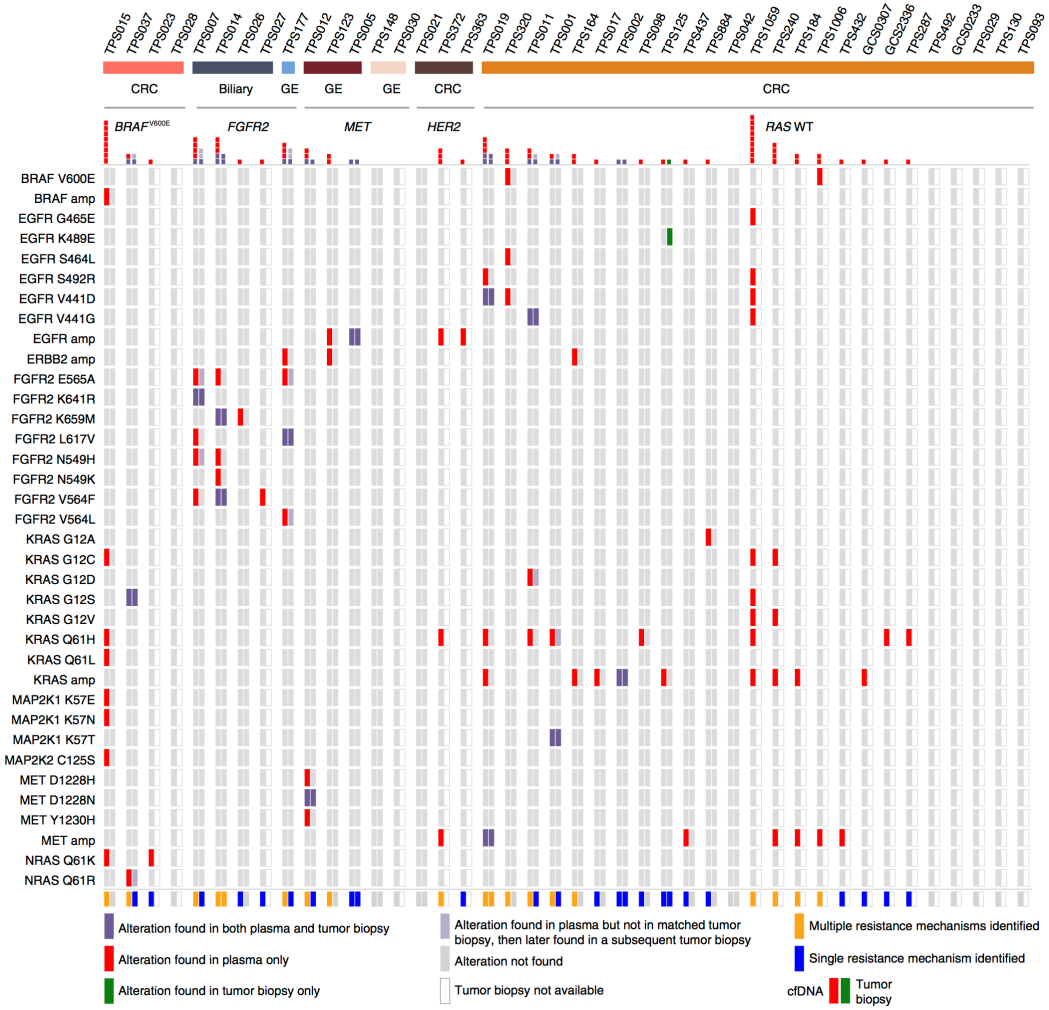
Evaluating gene expression change is difficult, but high/low levels of specific genes is feasible

Different tissues activate specific transcriptional programmes, this approach may be used to infer tissue of origin

Ulz et al, Nature Communications 2019

How about somatic alterations? Can ctDNA replace a tissue biopsy?

Evidence suggest ctDNA is effective for molecular characterisation, and may be robust to *intratumour heterogeneity*!



- 42 patients with gastric cancer
- Acquired resistance to targeted therapy
- Gene panel (Guardant 360, 74 genes)
- ctDNA identifies resistance mutations

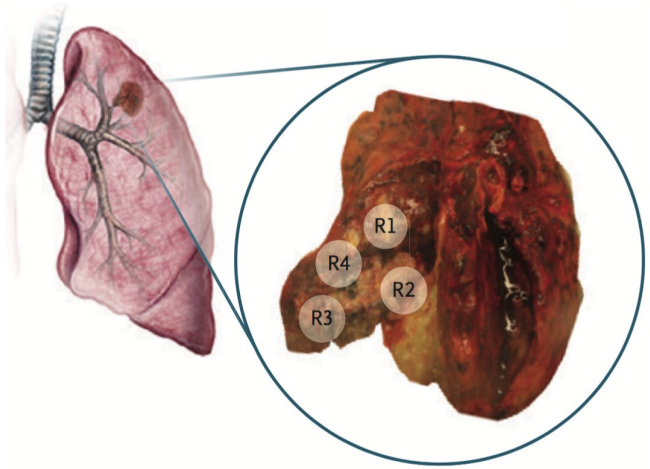
Corcoran et al, Nature Medicine 2019

Intratumour heterogeneity and longitudinal ctDNA tracking of cancer evolution

- Cancer evolution is continuous, both before and after treatment
- We can biopsy and characterise a tumour, but how do we track the status of the evolving disease during and after therapy?
- **Using phylogenetic analysis & longitudinal ctDNA tracking**

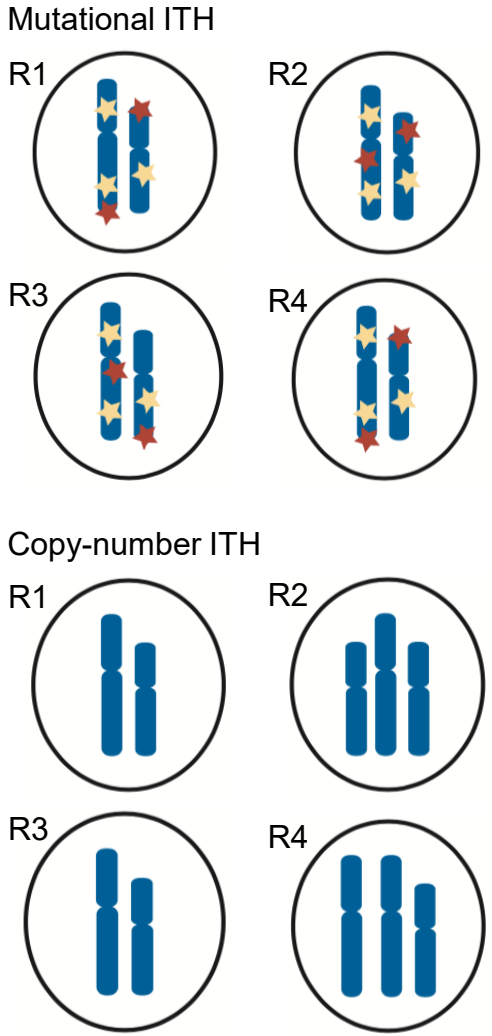
TRACERx: Tracking Cancer Evolution through Therapy

Multi-Region Sequencing

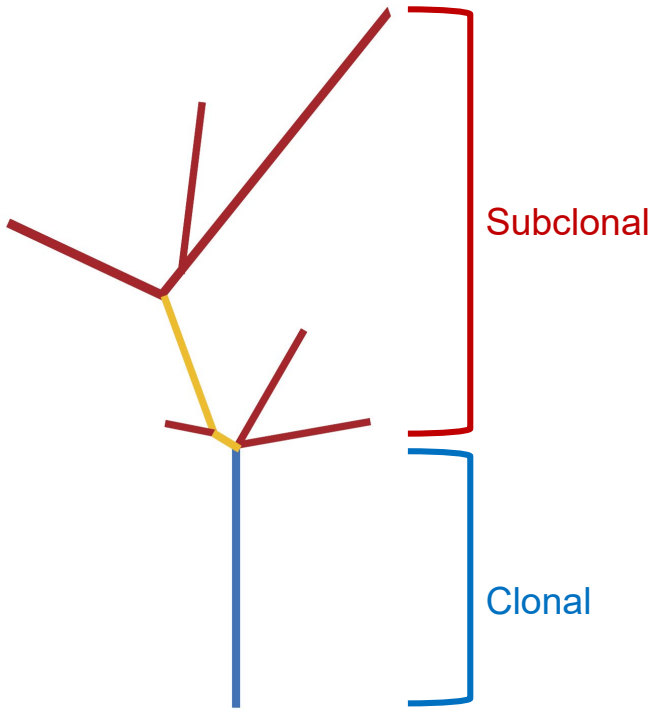


- Whole-Exome Seq
- Bulk RNA-seq

Assessment of Intra-Tumour Heterogeneity

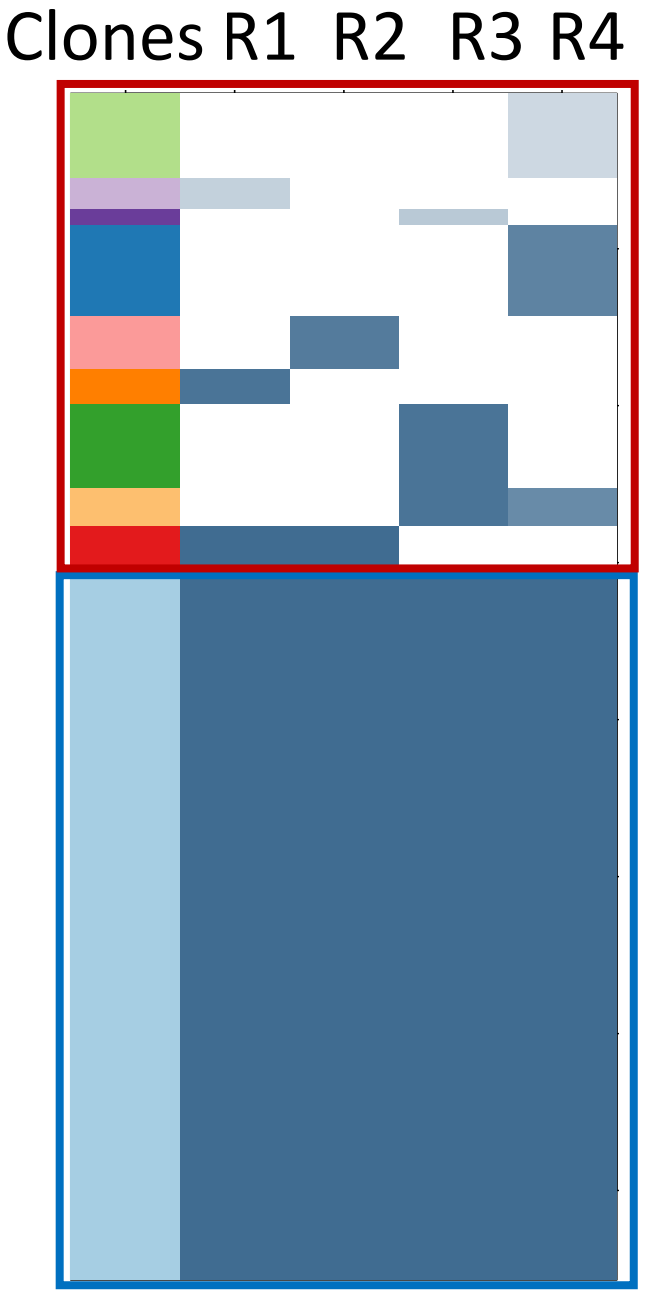


Timing of Somatic Events in Cancer Evolution



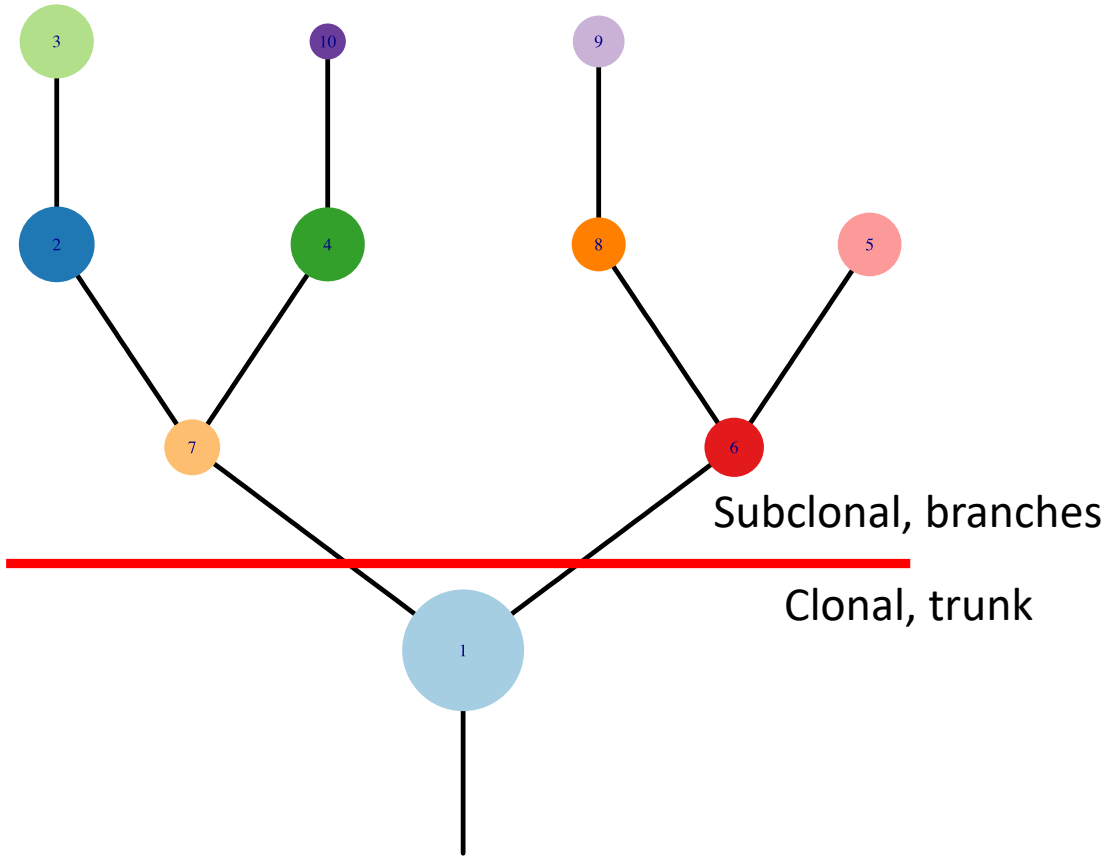
Jamal-Hanjani, NEJM 2017

Using mutations to infer phylogenetic relationship and clonality

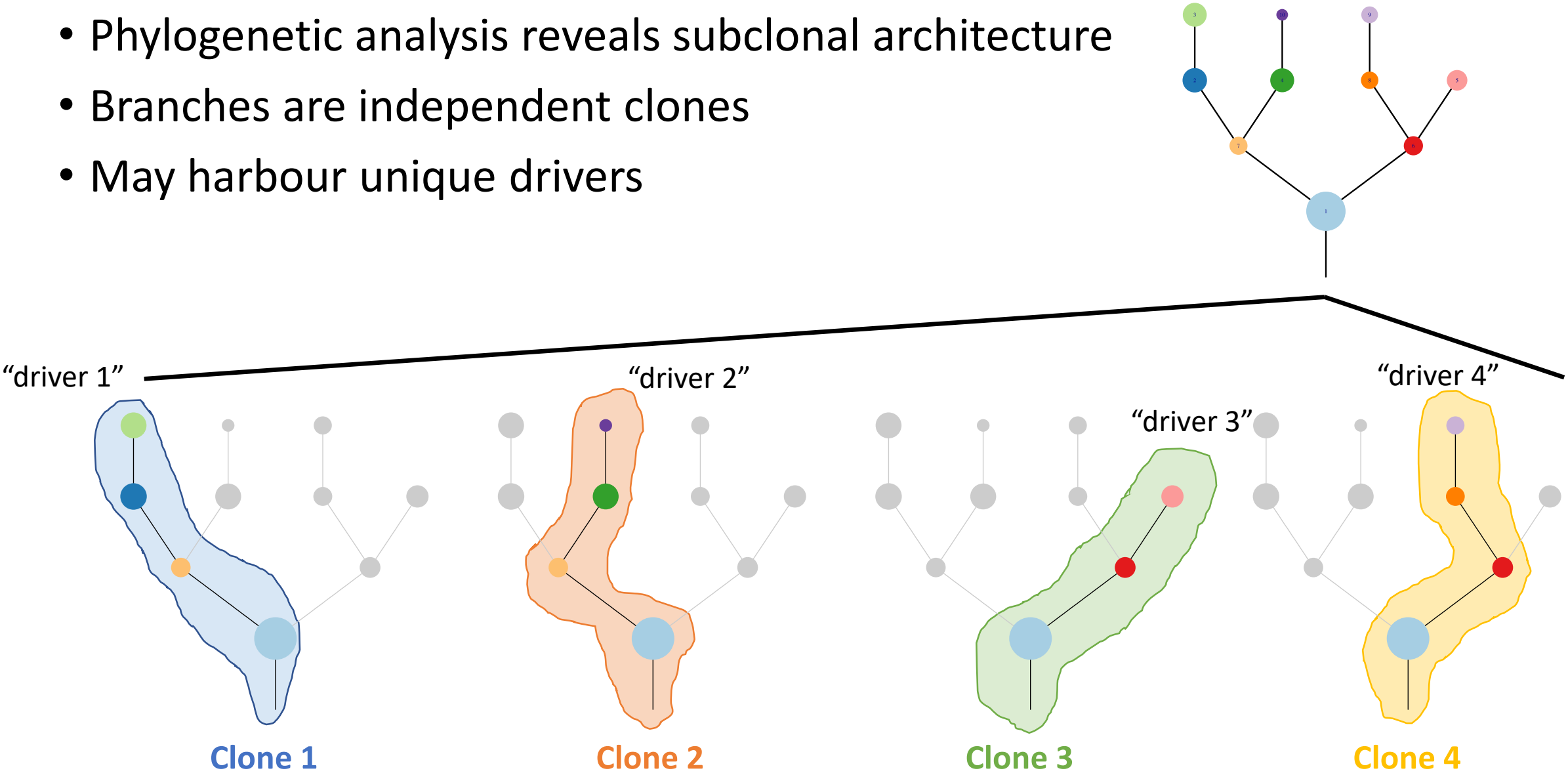


Subclonal mutations
"The Branches"

Clonal mutations
"The Trunk"

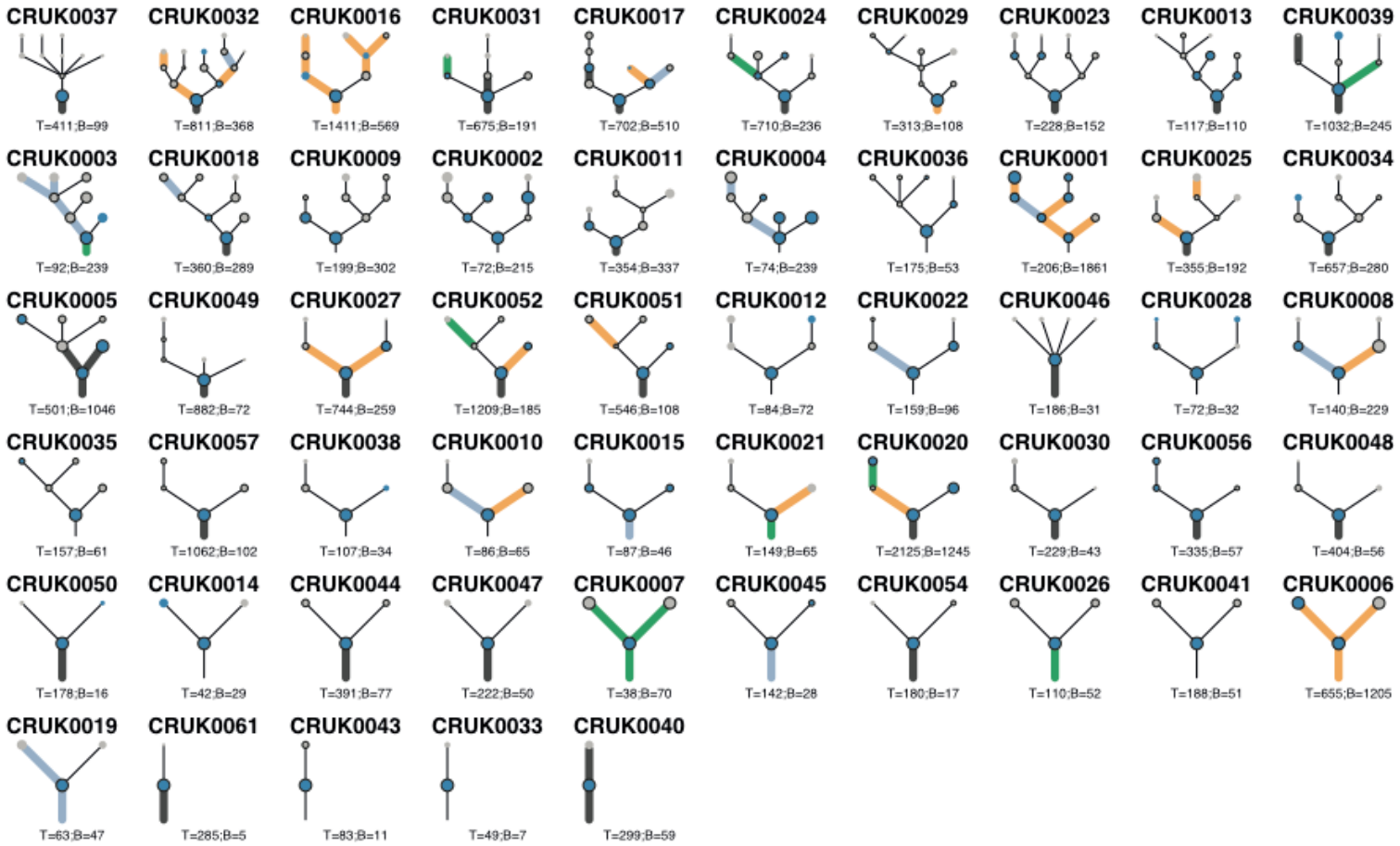


- Phylogenetic analysis reveals subclonal architecture
- Branches are independent clones
- May harbour unique drivers



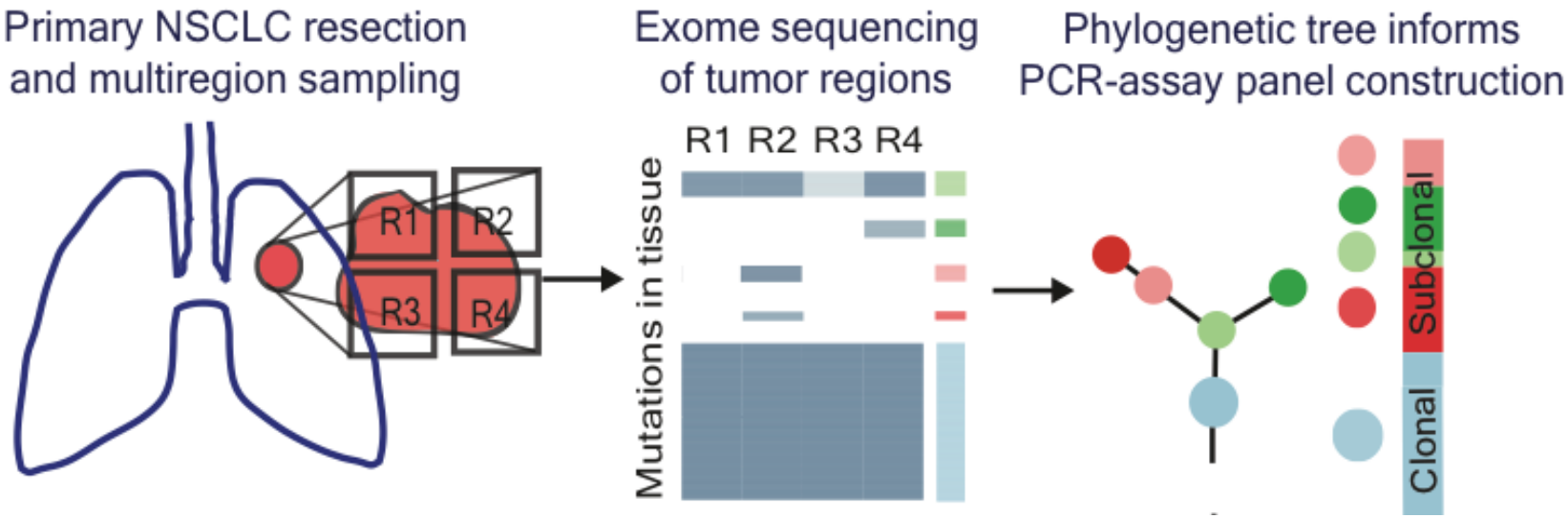
Lung cancer tumours highly heterogeneous

- Some tumours show extensive intratumour heterogeneity
- Subclonal drivers may define metastatic disease
- How do we track which subclone drives disease relapse?



Jamal-Hanjani et al. NEJM (2017)

Bespoke multiplex PCR NGS ctDNA profiling



Abbosh et al. Nature (2017)

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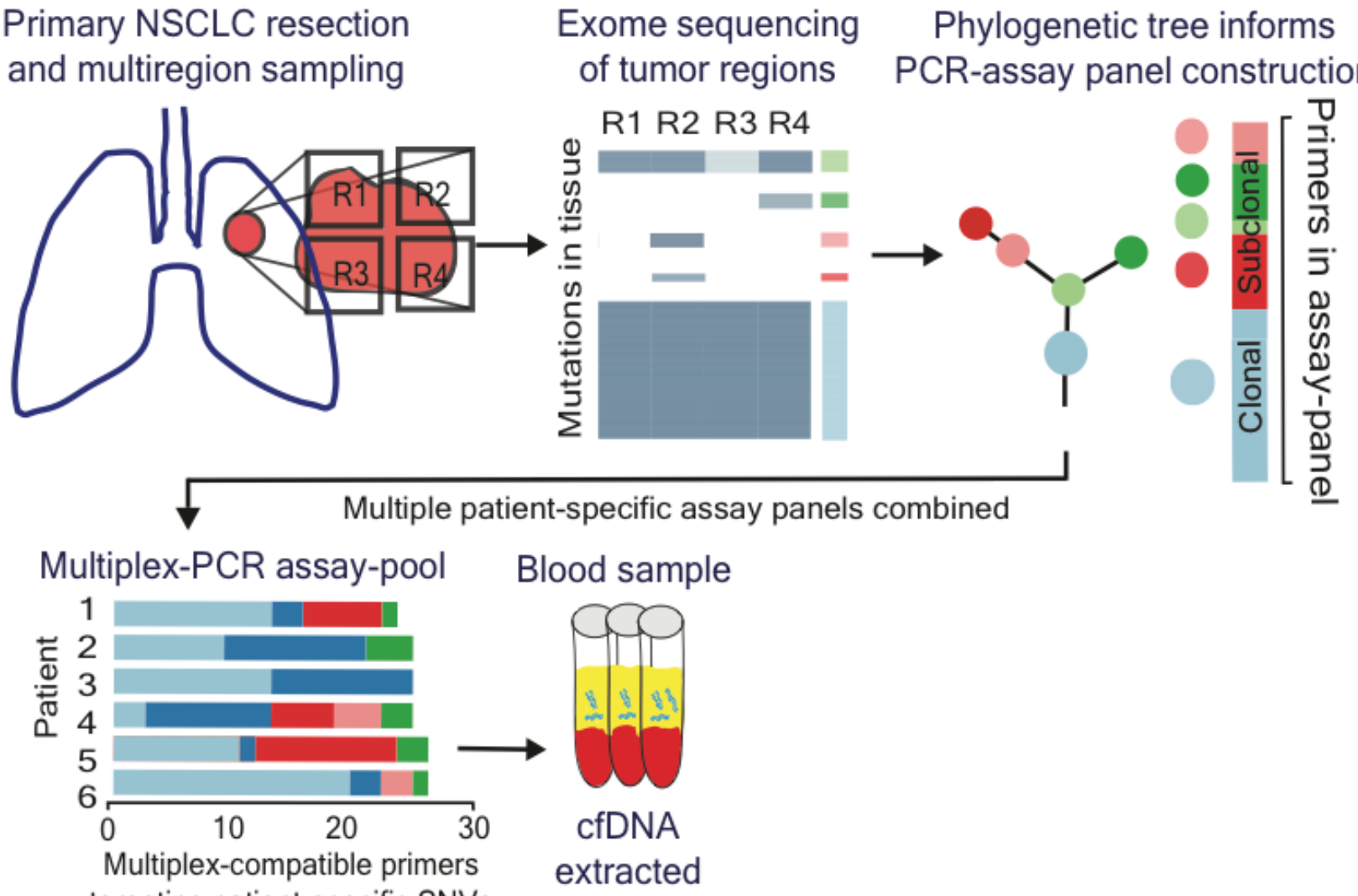

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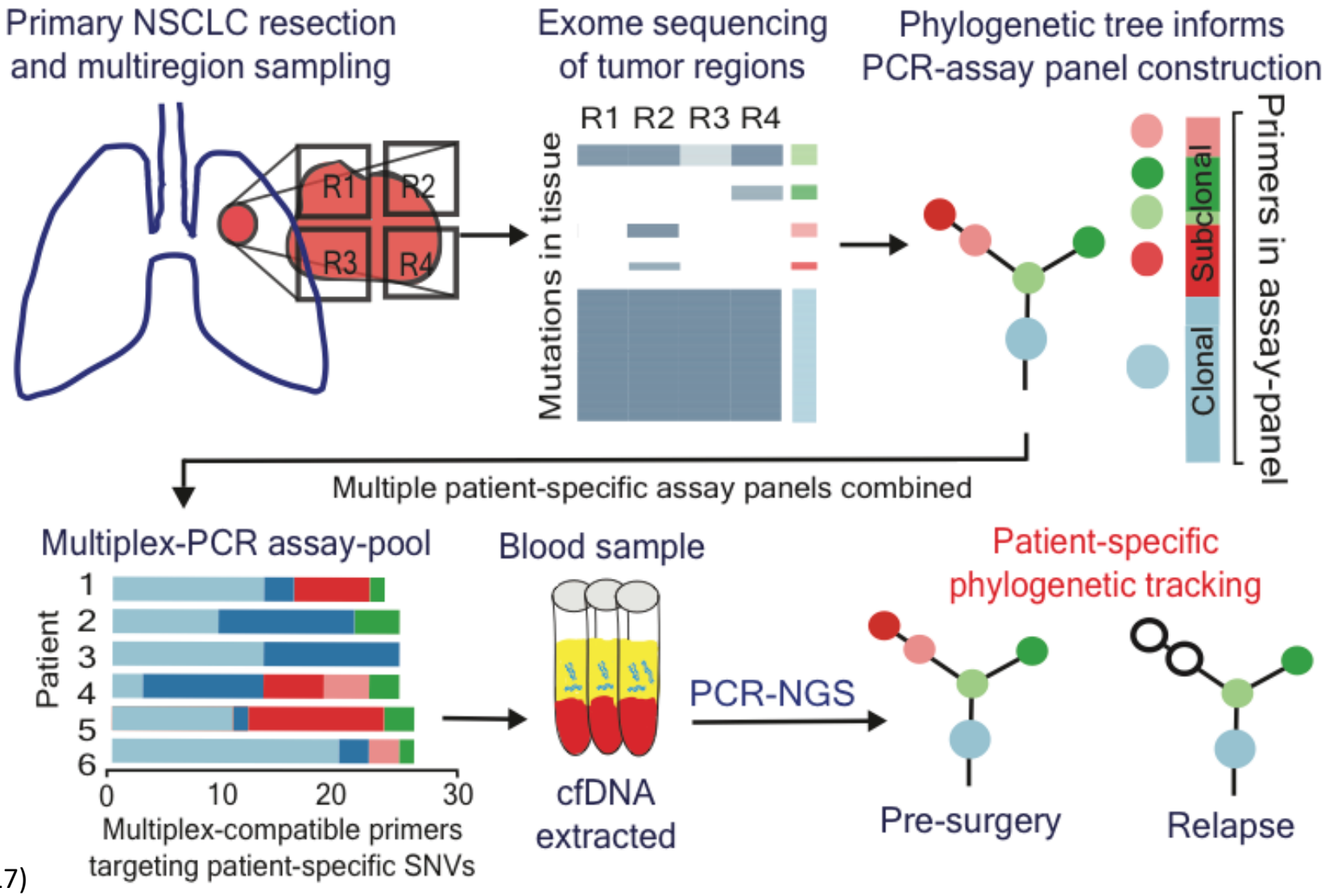
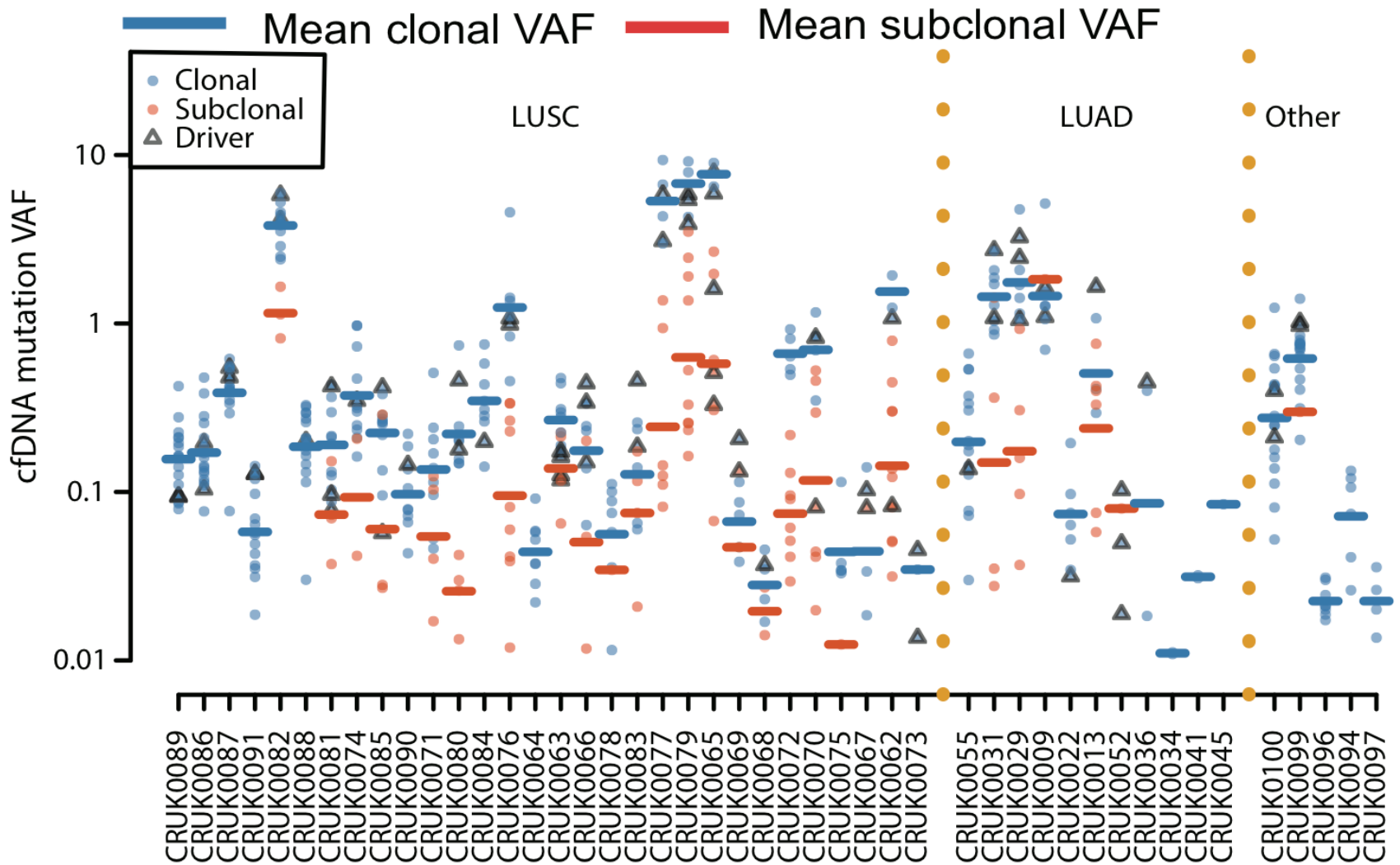
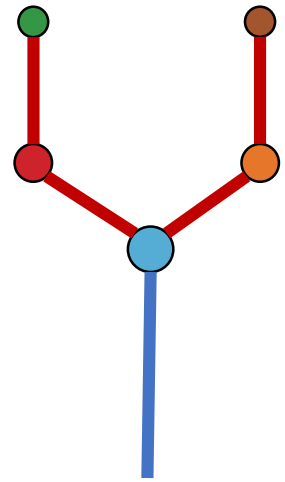


Abbosh et al. Nature (2017)

Clonal SNVs show higher VAF

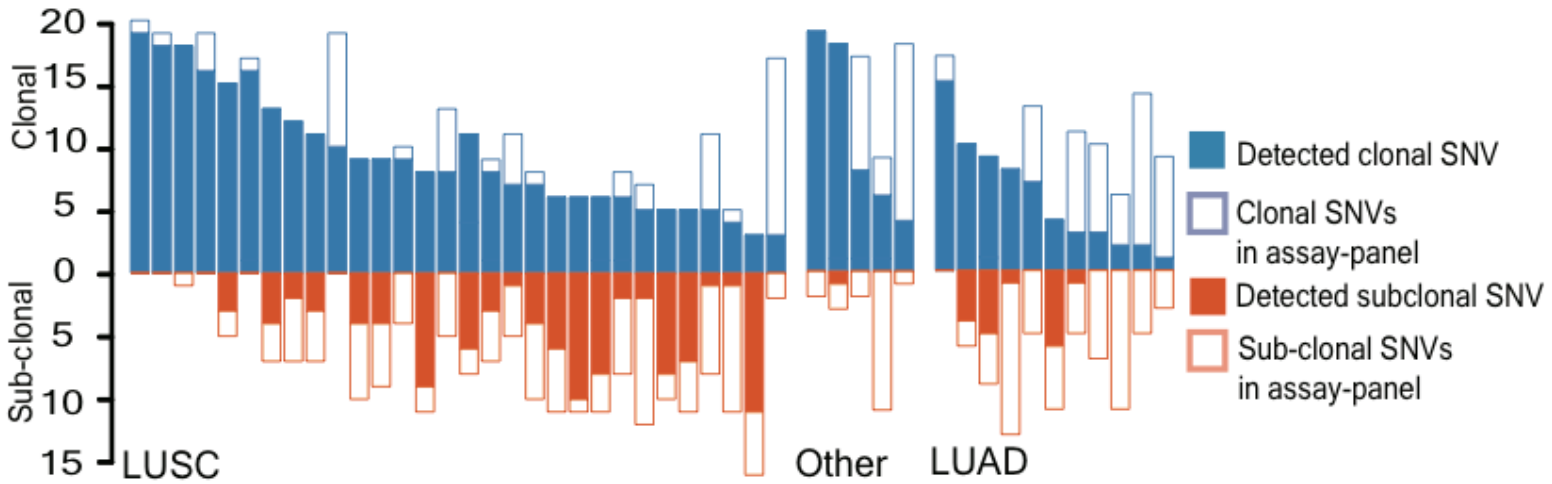


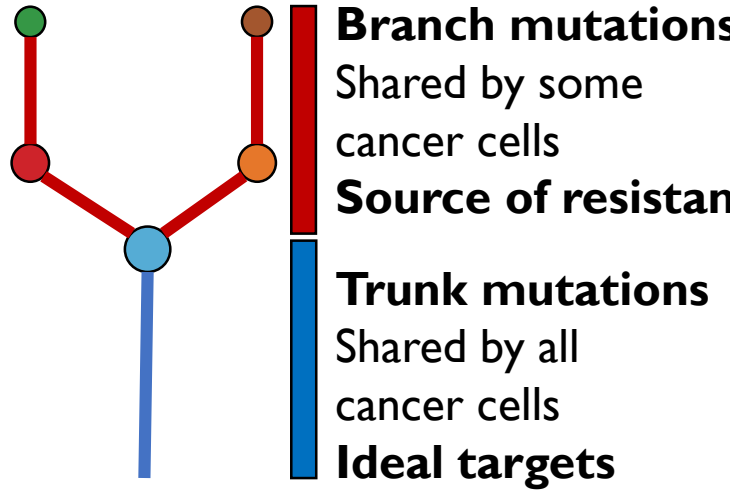


Branch mutations
Shared by some cancer cells
Source of resistan

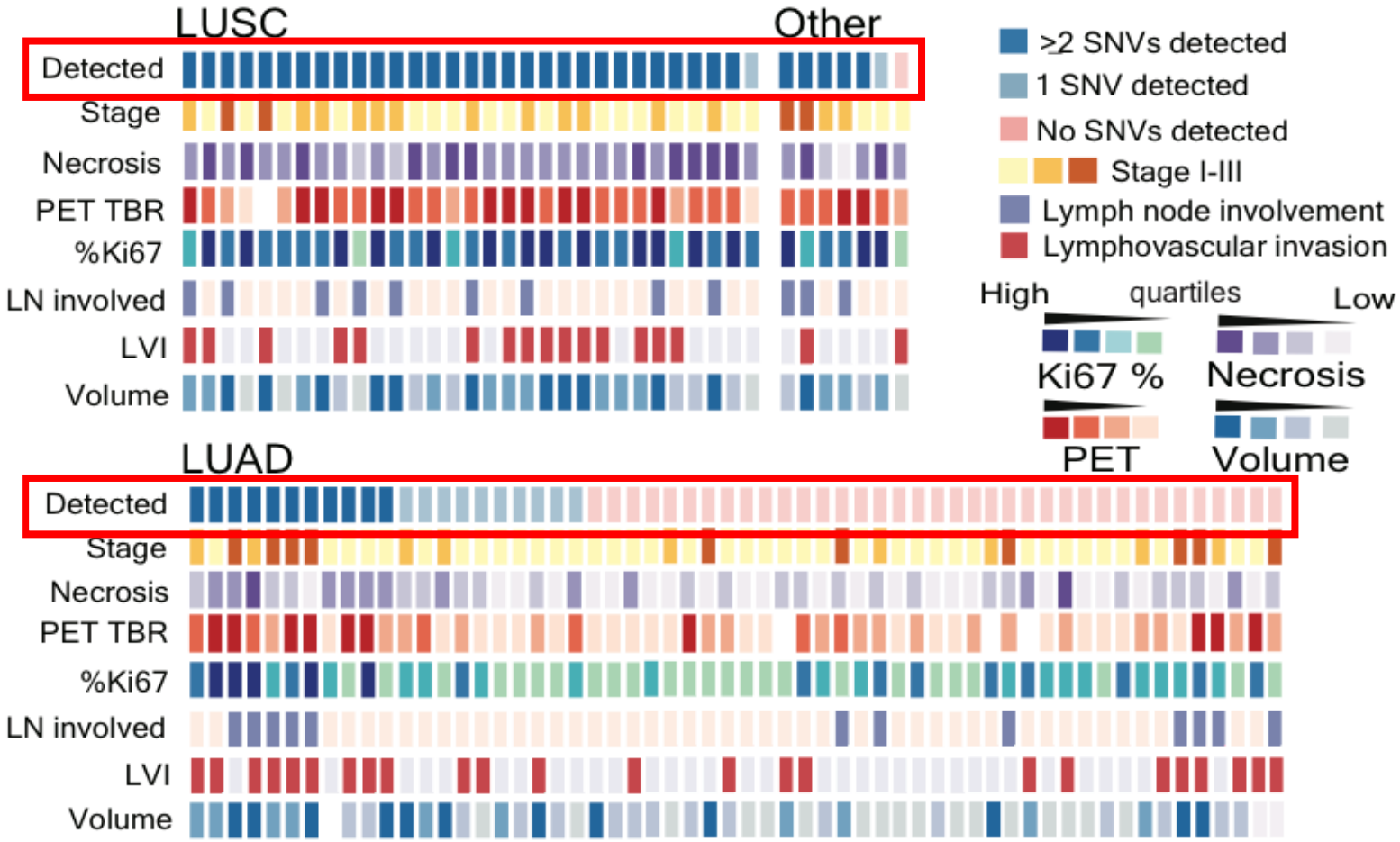
Trunk mutations
Shared by all cancer cells
Ideal targets

Clonal SNVs easier to detect compared to subclonal at baseline





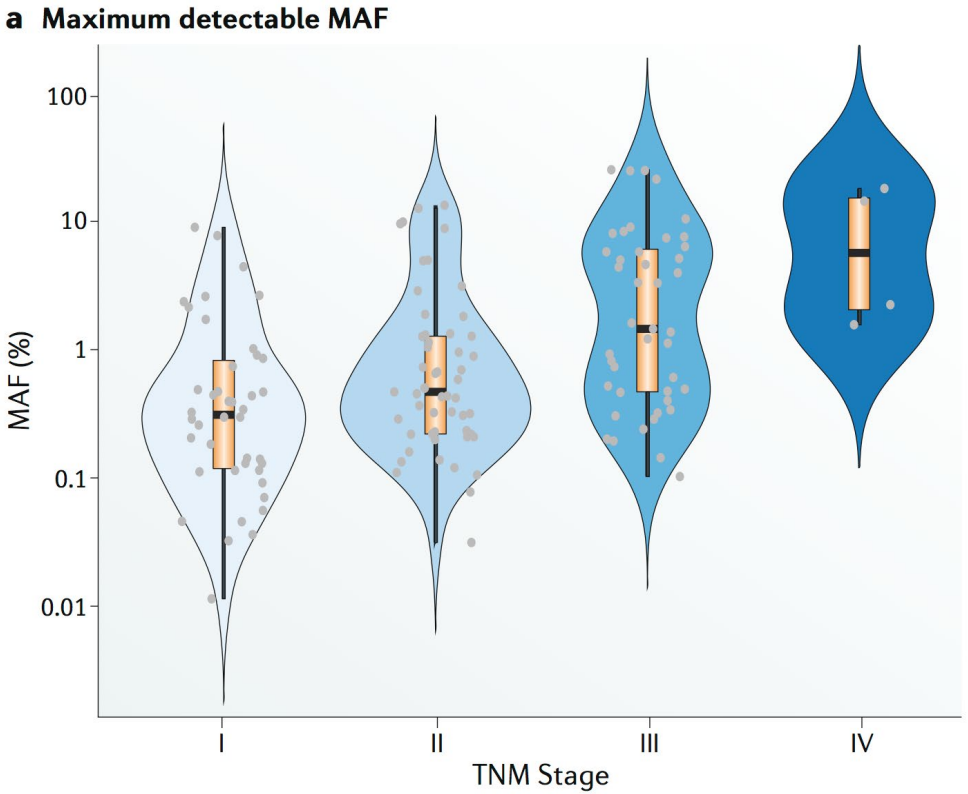
Analysis performed on 96 lung cancer patients 48% of cases detected by ctDNA, including almost all squamous non-small cell lung cancer (NSCLC LUSC)



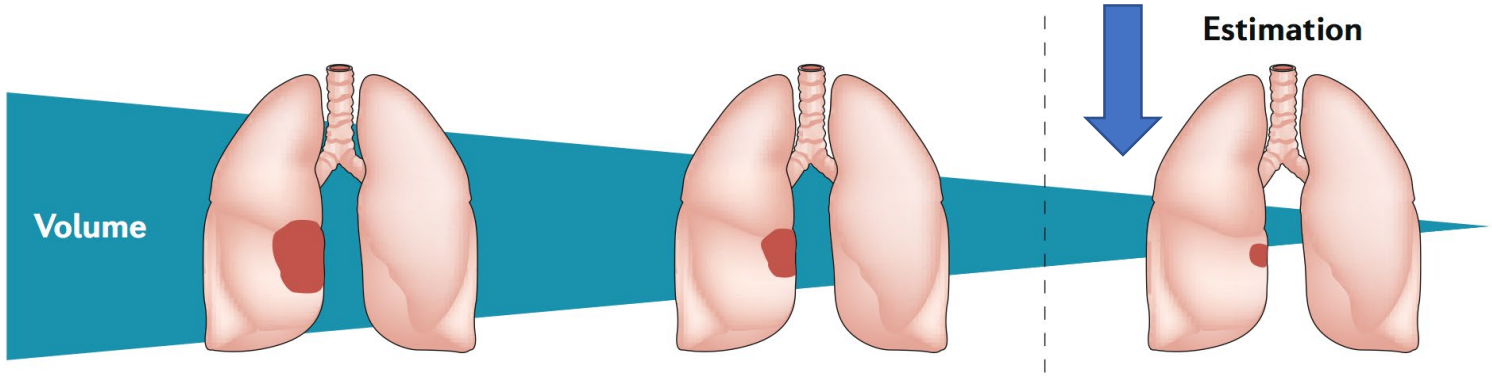
Of lung adenocarcinoma, only 11/58 were detected with ctDNA at baseline (prior to surgery)

Not all patients with cancer have detectable ctDNA

Tumour burden correlates with ctDNA amount in plasma



ctDNA assay limit of detection

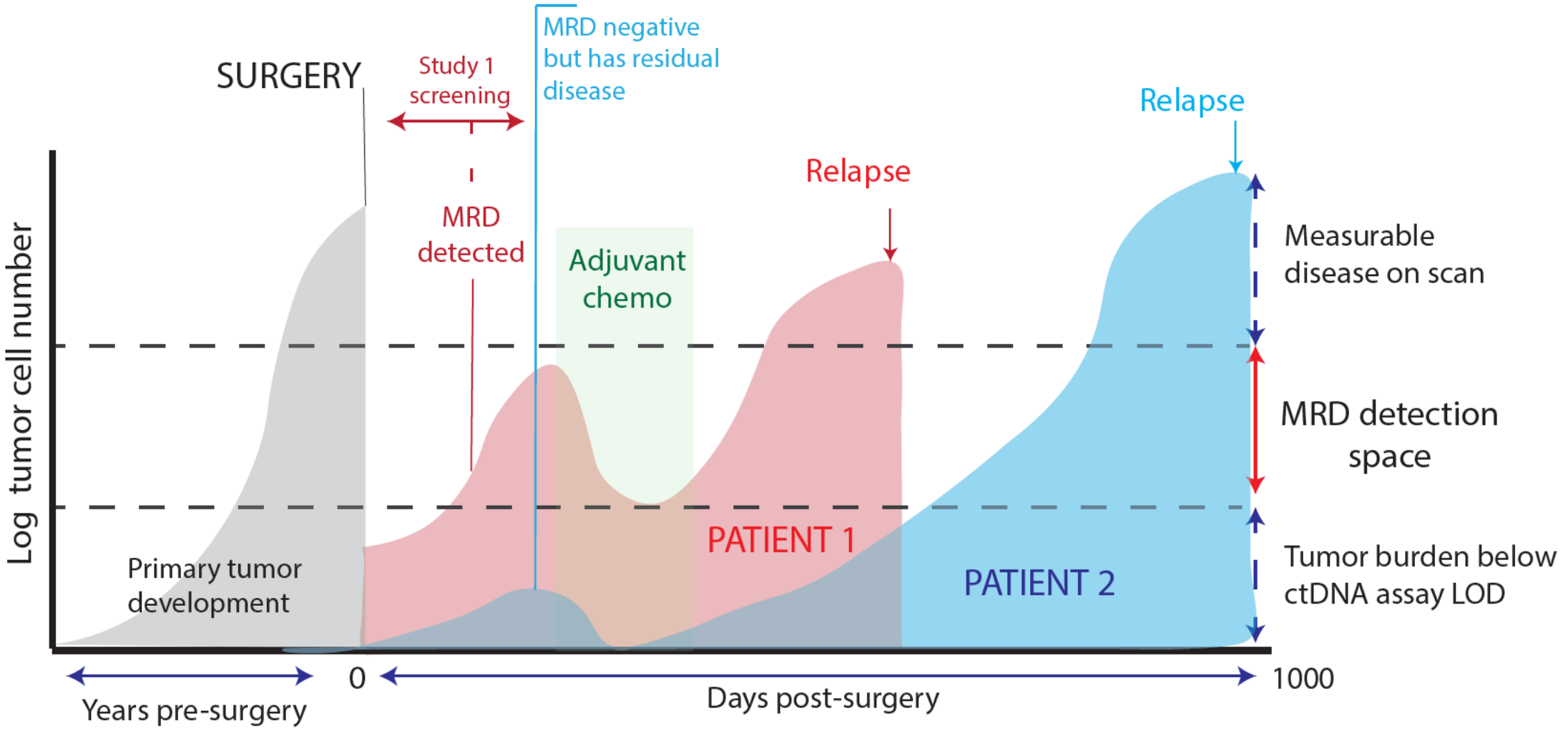


MAF	1.4% (0.62–3.1%)	0.1% (0.06–0.18%)	0.008% (0.002–0.03%)
Nodule diameter	5.8 cm	2.6 cm	1.2 cm
Nodule volume	100 cm^3	10 cm^3	1 cm^3
T stage	T3	T1c	T1b

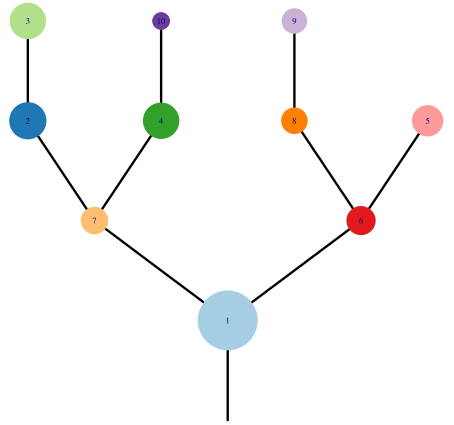
Abbosh, Birkbak Nature reviews Oncology 2018

Not all patients with cancer have detectable ctDNA

ctDNA assay limit-of-detection will limit MRD prevalence



Phylogenetic tracking

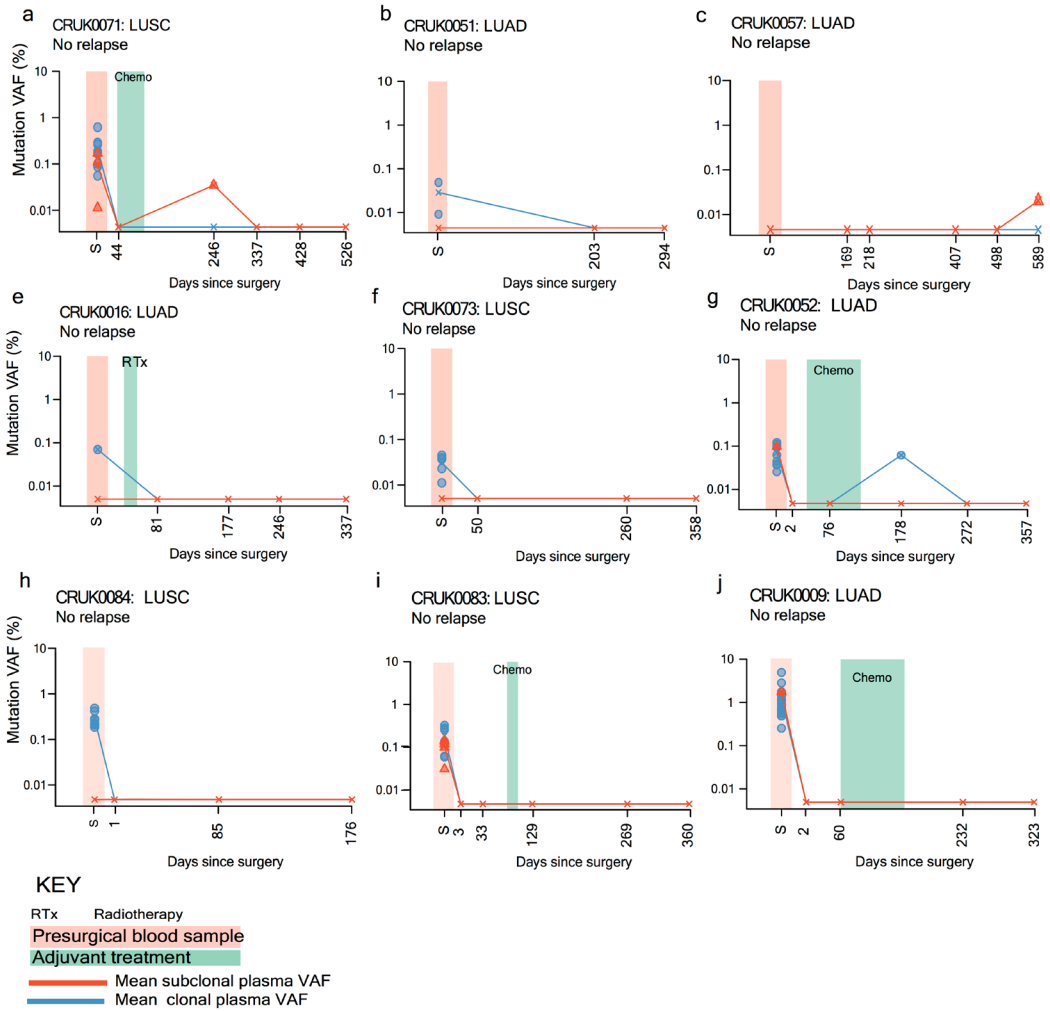


- Tracking metastatic tumor evolution through the nodes in the phylogenetic tree

- 24 patients tracked longitudinally
- 12 relapse, 12 controls (median follow-up for controls, 775 days)
- 2 controls relapsed during study
- Median lead-time was 70 days before confirmed by CT-scan (range 10- 346)

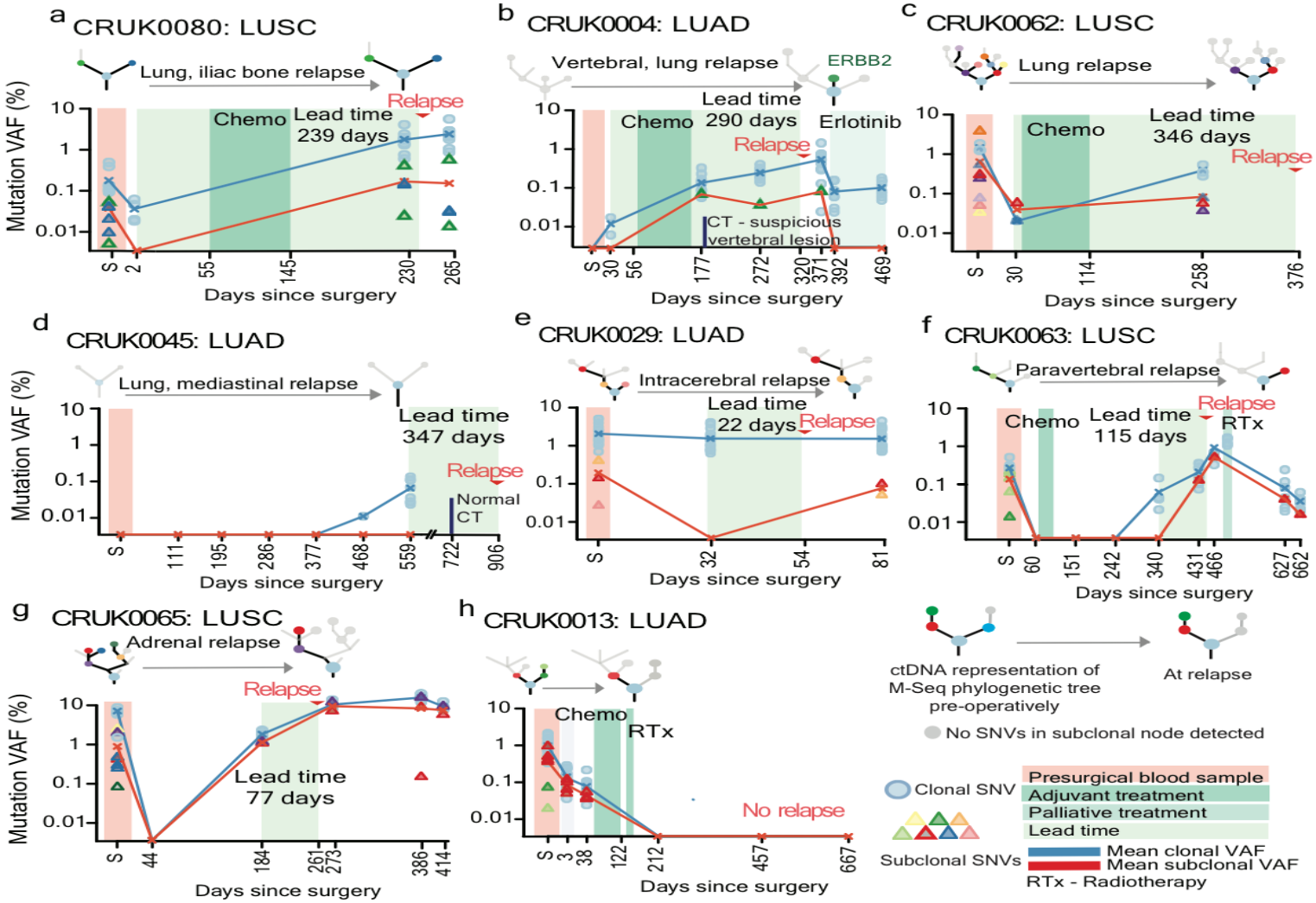
Tracking tumor clones in control cases shows rapid loss of detection of SNVs

Control cases

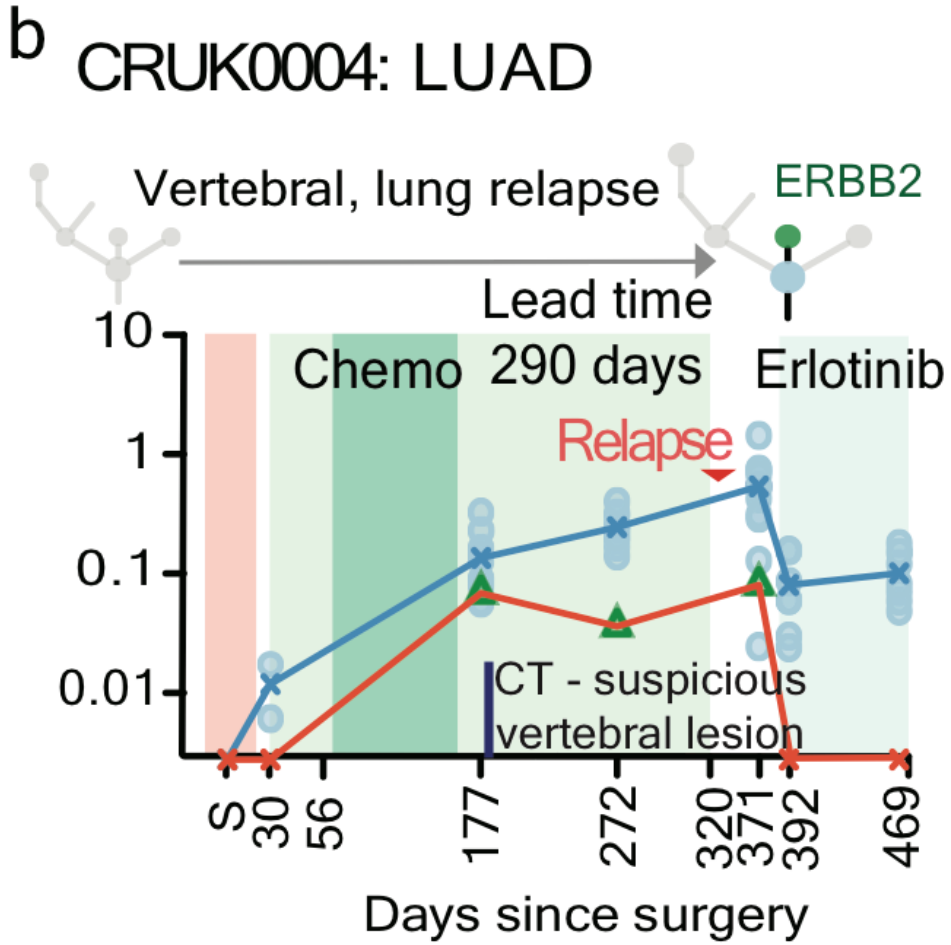


Tracked SNVs increase prior to confirmed relapse, phylogenetic tracking identifies the relapsing subclone

Relapse cases



Phylogenetic tracking via ctDNA allows early detection and identifies the relapsing subclone



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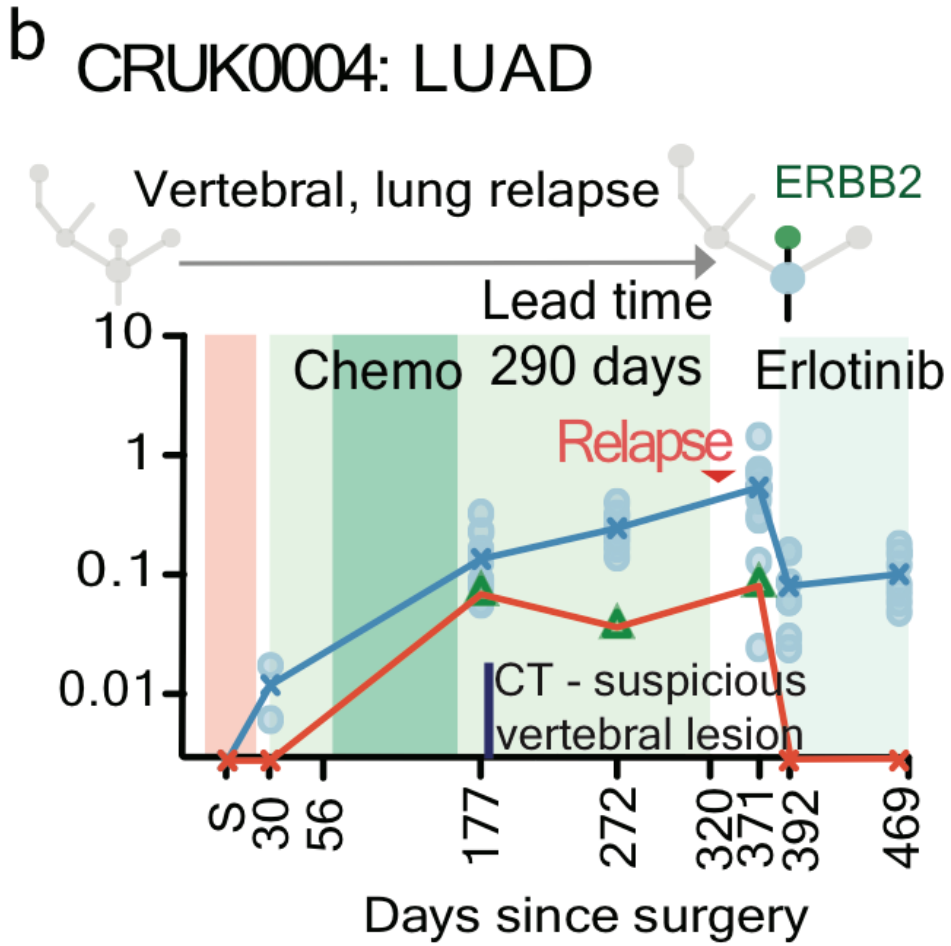
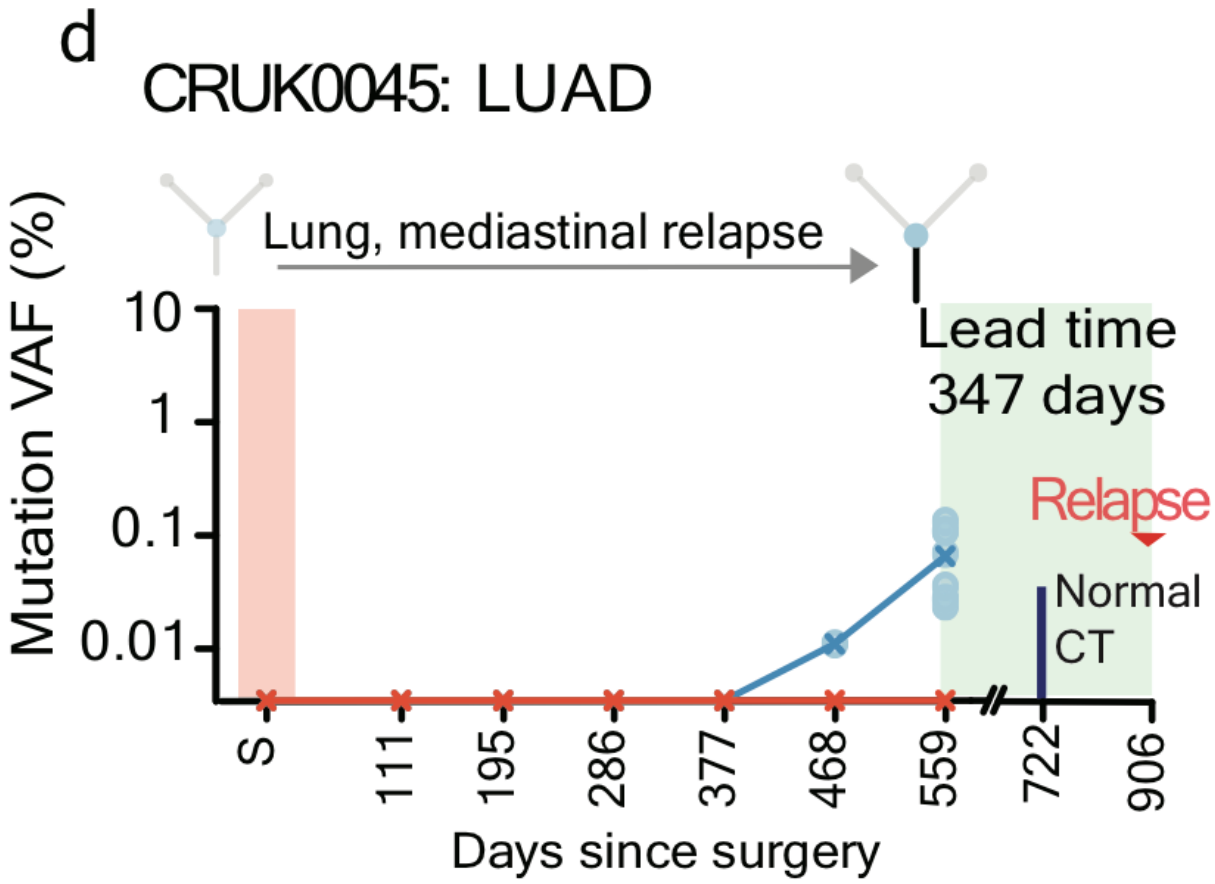
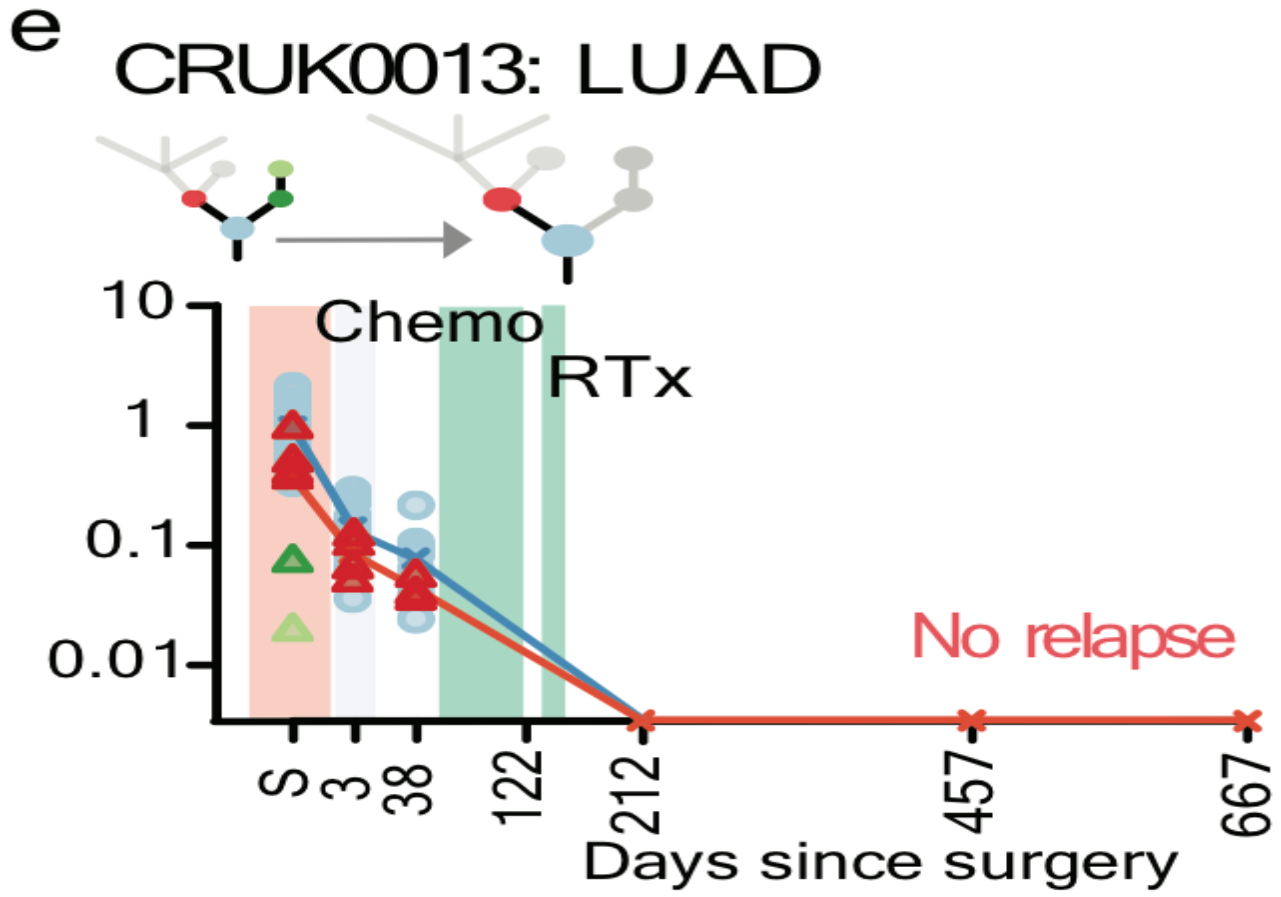
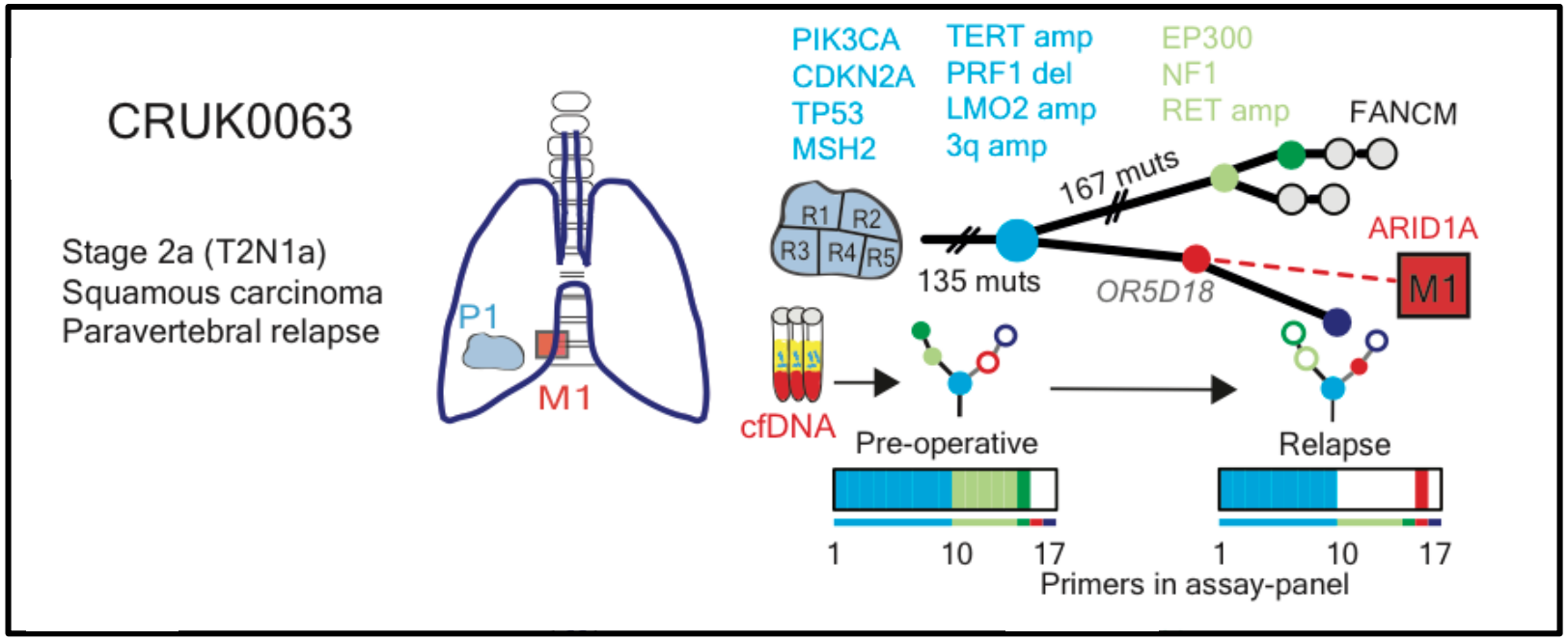


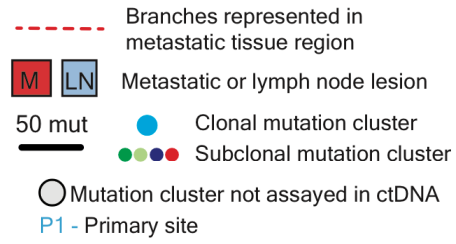


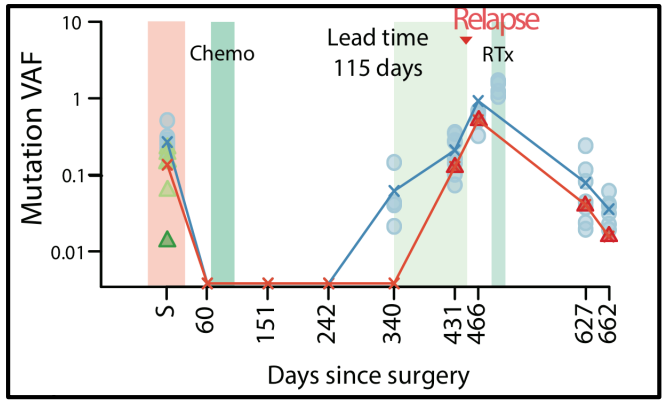
ctDNA tracking also shows residual disease – and the effect of adjuvant therapy



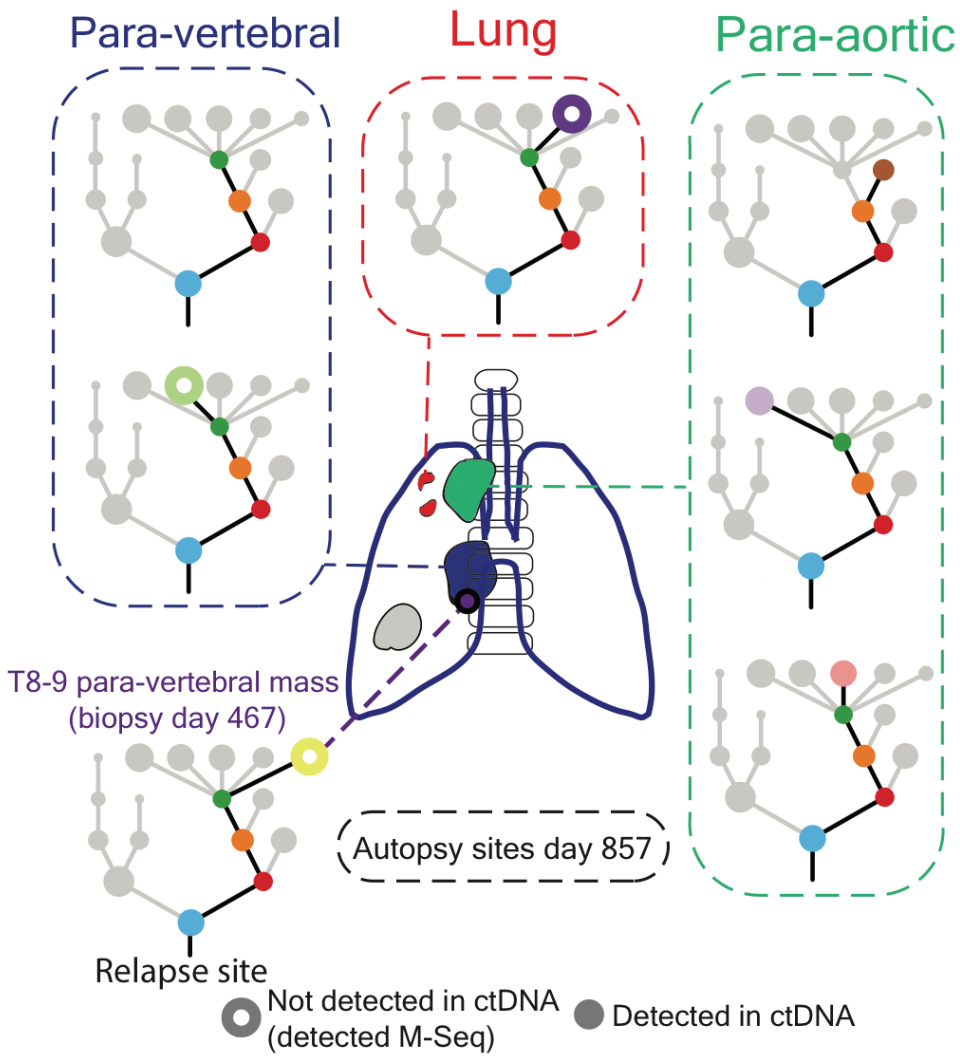
Minor subclone from CRUK0063 primary caused relapse, death







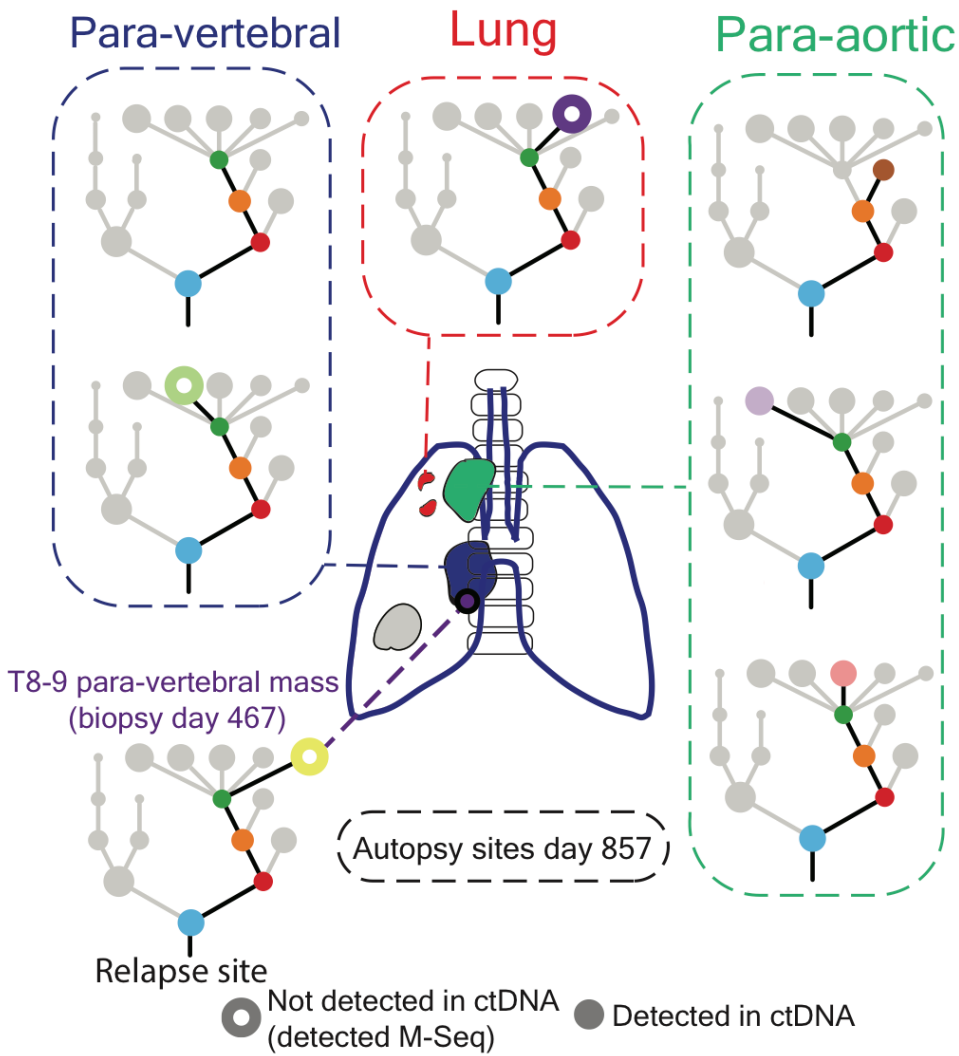
CRUK0063 was recruited to PEACE – a fast autopsy program

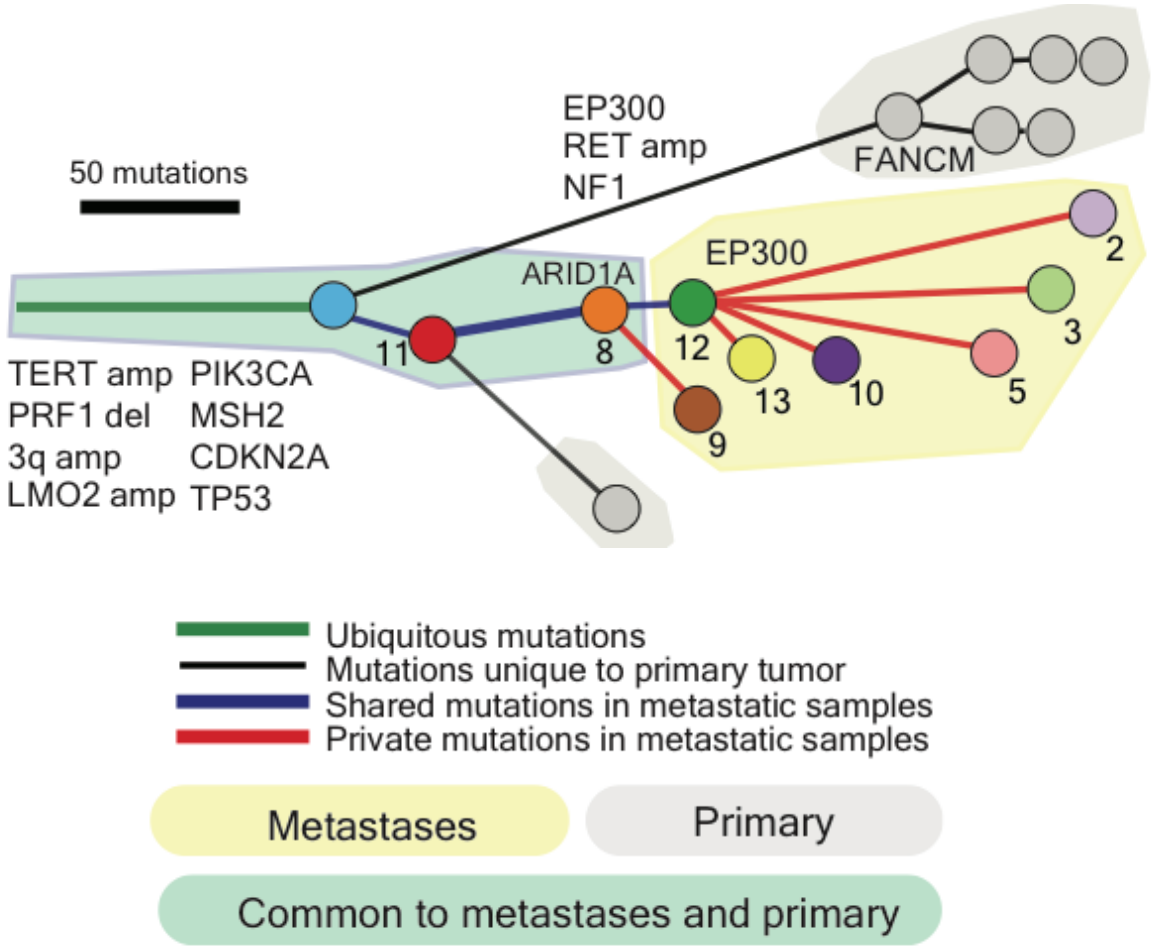


- CRUK0063 subjected to autopsy within 24 hours of death
- Multiple metastatic lesions resected
- 6 tissue biopsies from 3 sites subjected to multiregion deep whole exome sequencing

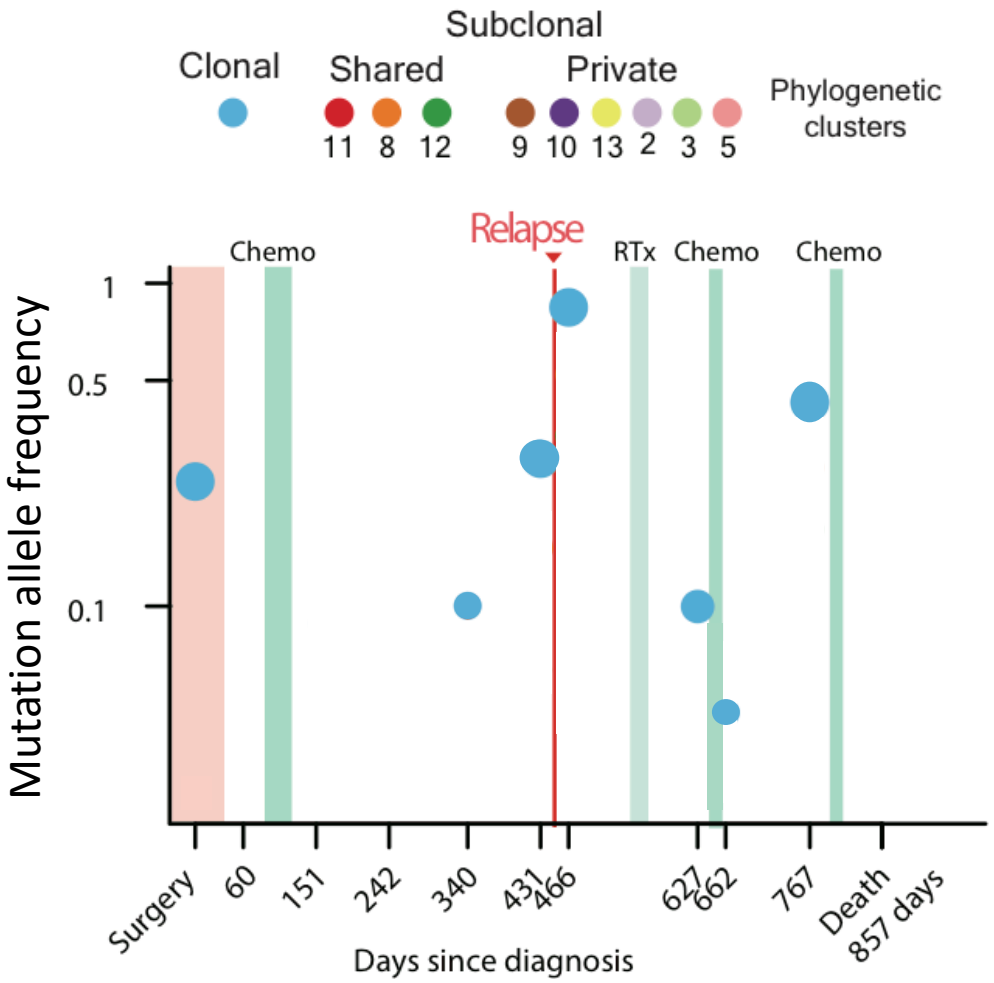
- Metastatic regions re-analysed with primary tumour regions and relapse biopsy

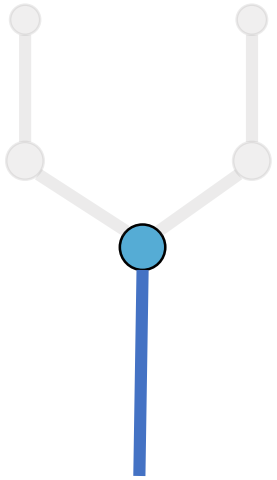
Phylogenetic tree revealed likely monophyletic spread





ctDNA profiling identifies **Trunk** mutations from **Branch** mutations





Trunk mutations
Shared by all cancer cells
Ideal targets

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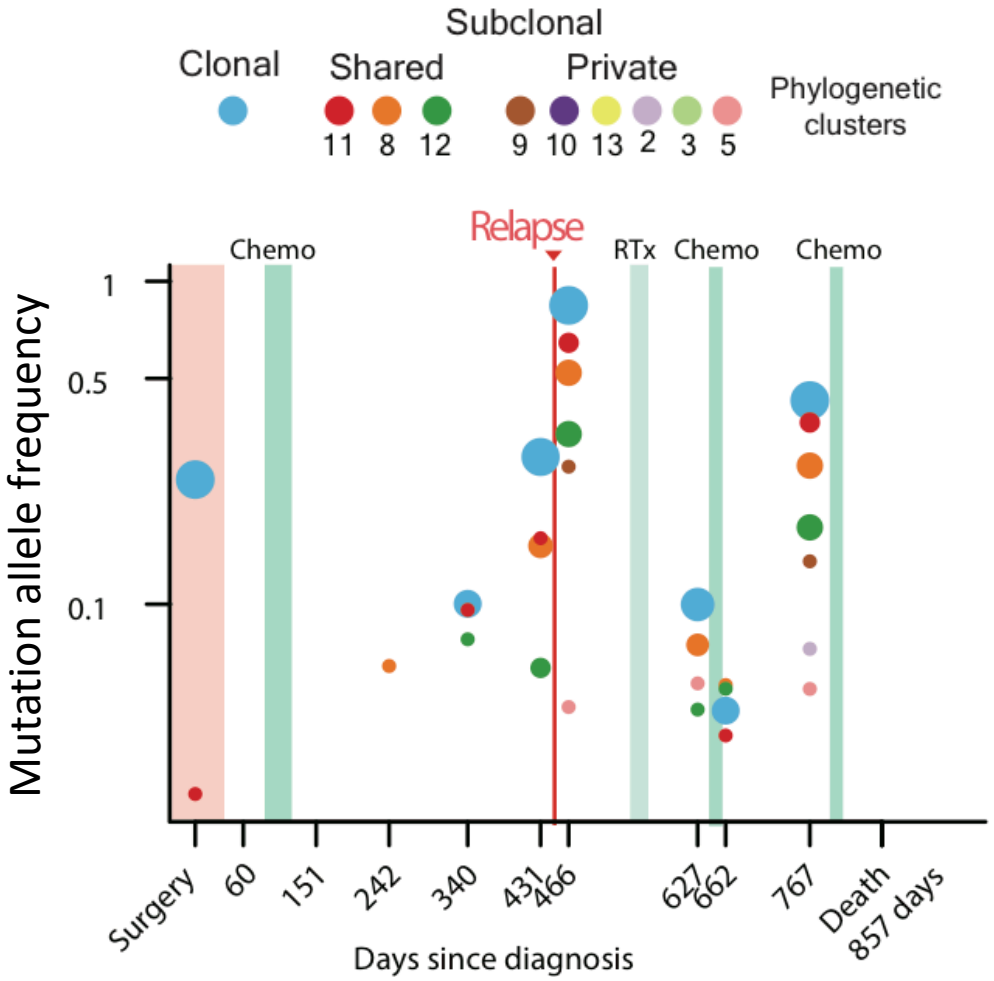

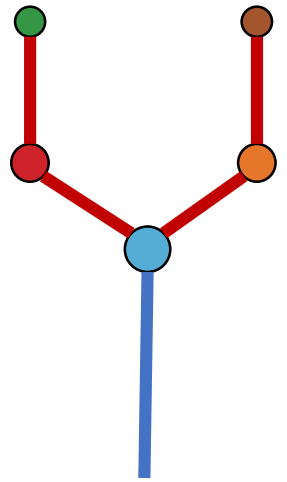

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Shared by some cancer cells
Source of resistance

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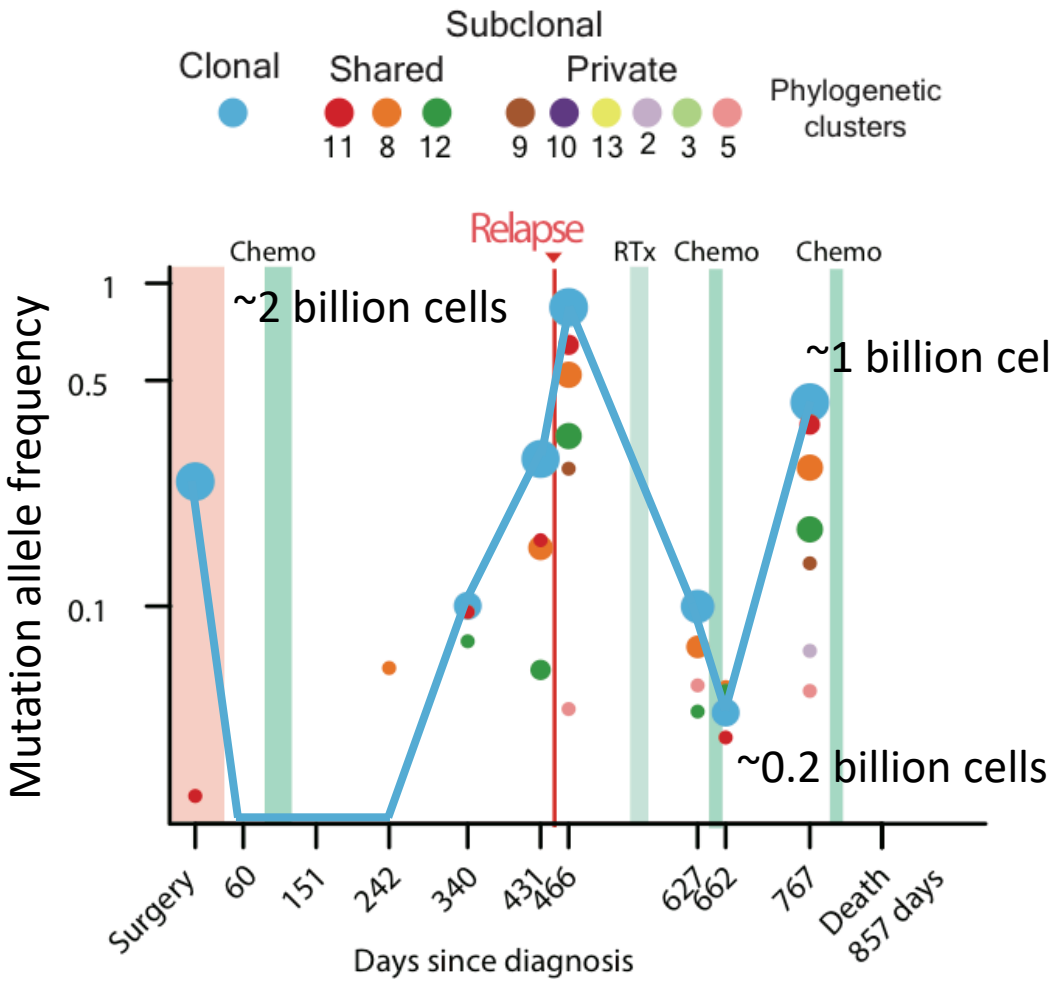
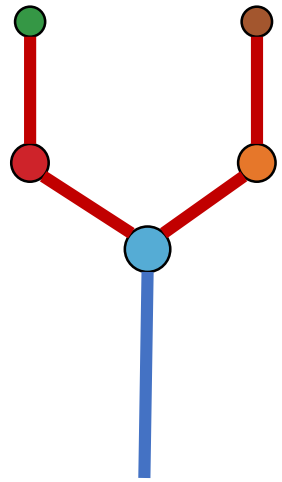
Trunk mutations in ctDNA **monitors cancer growth** and **drug sensitivity**





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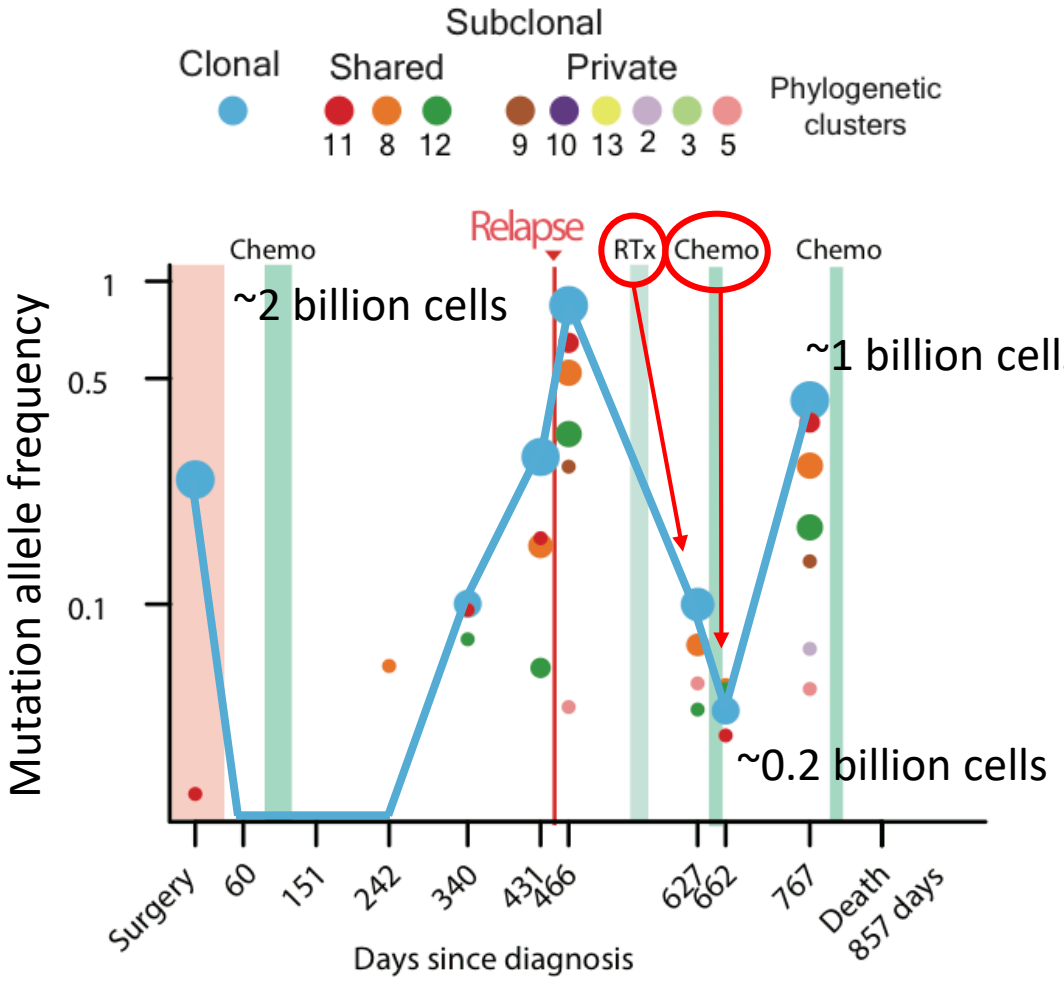

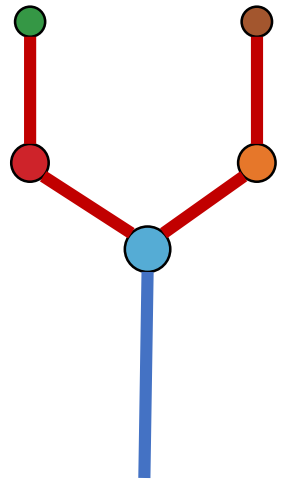

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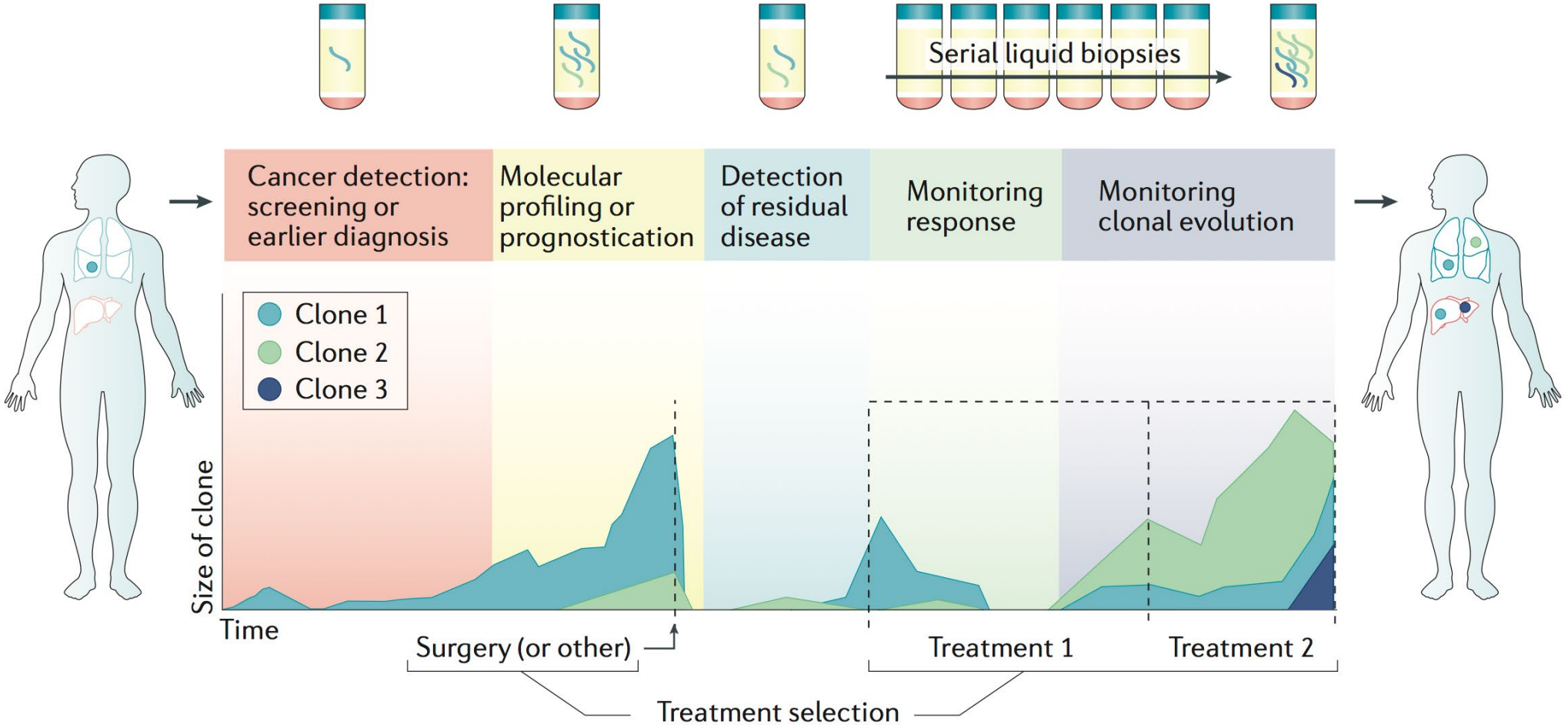
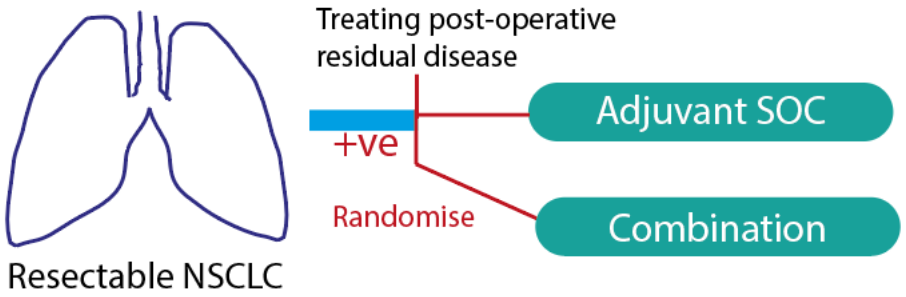
Summary
ctDNA to decide treatment and disease tracking



Wan, Nature Reviews Cancer, 2017

ctDNA as a pre-adjuvant MRD biomarker:



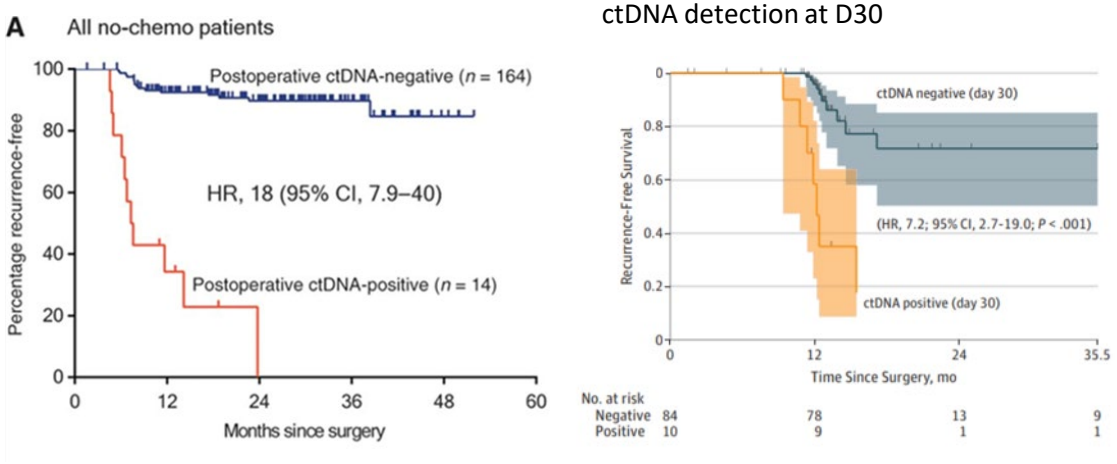
Advantages:

- Enrich for small populations with low DFS and high-event rate – targets for combination therapy?

Limitations:

- Biological constraints (e.g. metastatic dormancy)?
- Large number of patients to adequately power interventional studies (high-screen failure rate).
- Logistical considerations to return result before adjuvant SOC decision especially with personalised panels.

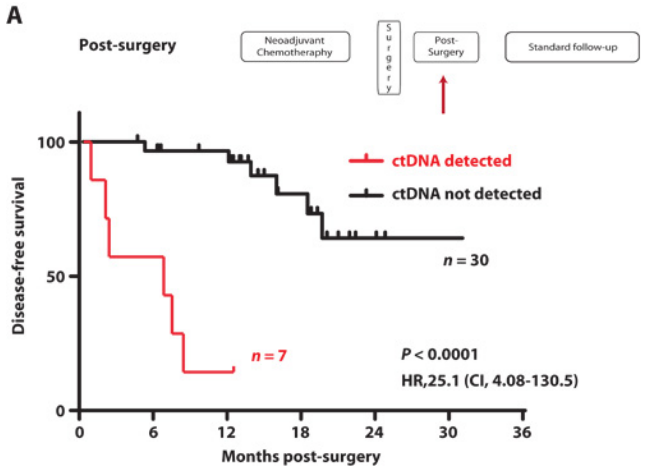
Colorectal cancer



Tie J, STM 2016

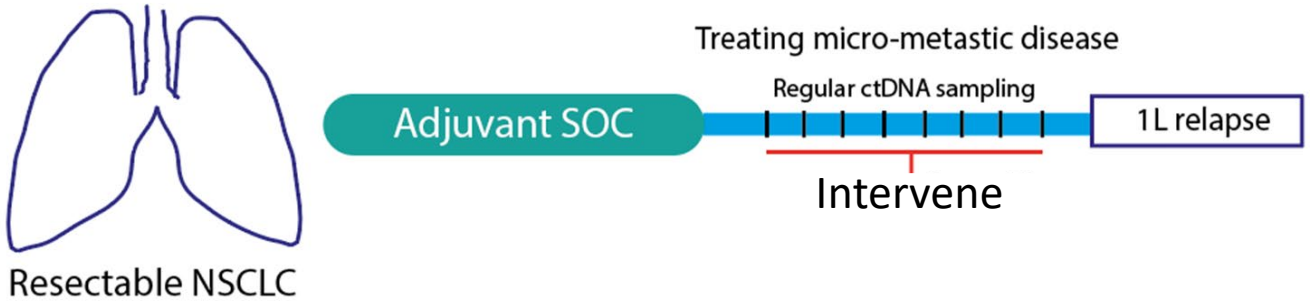
Reinert T, JAMA 2019

Breast cancer



Garcia-Murillas I, STM 2015

ctDNA as a post-adjuvant MRD biomarker:

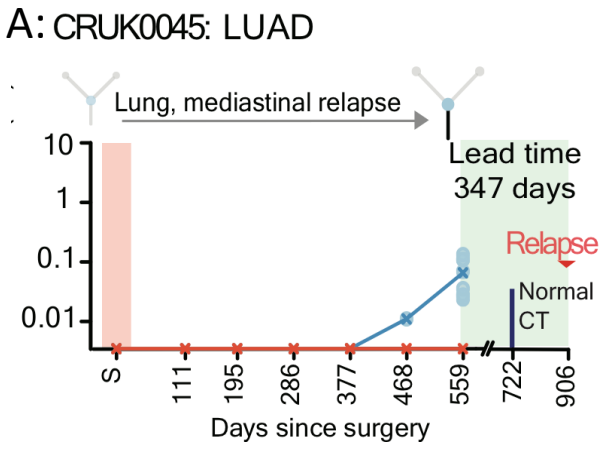


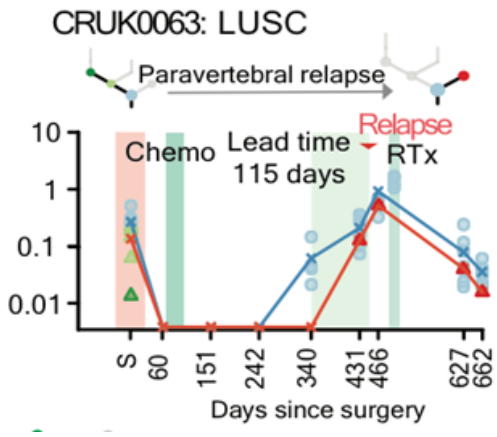
Advantages:

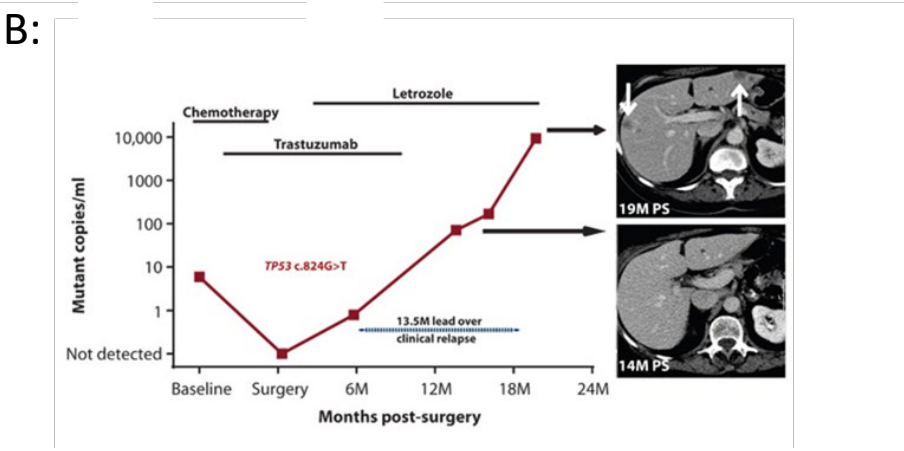
- larger proportion of DFS events across a population identified.
- ctDNA monitoring feasible at frequencies exceeding imaging [facilitates intervention at small disease volumes].
- Decreases screen failure rate

Disadvantages:

- Translatable into routine practice, relationship with surveillance imaging?







A: Abbosh et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution, Nature 545, 2017

B: Garcia-Murillas I, Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer, Sci Transl Med. 2015 Aug 26;7(302)

Main take-away

- ctDNA has immediate utility in early relapse detection
- ctDNA may be used for molecular characterisation
  - Improved diagnosis
  - Identify tissue of origin
  - Overcome intratumour heterogeneity
- Phylogenetic tracking reveals lethal metastatic clone, metastatic disease dynamics and cancer evolution

Discussion points

- Why are clonal mutations easier to detect? Are there other specific mutations that might be better to track?
- When is phylogenetic tracking of relapse relevant? Does it depend on cancer type?
- Consider clinical trial settings for cancer drugs. Expensive, requires lots of patients. What are the potential benefits of incorporating ctDNA here?