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BIOTECH RESEARCH & INNOVATION CENTRE (BRIC)
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Tracing clonal evolution in brain tumors following treatment

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1. Background

- **Glioblastoma represents ~50% primaries malignant brain tumors**
- **Glioblastoma multiforme (GBM) is highly heterogeneous and aggressive: median surviaval 15 months**
- **Cancers evolve through progressive steps of mutation and selection, potentially resulting in multiple cell populations: intratumor heterogeneity (ITH)**
- **Understanding hte complex genomic patterns behind tumor progression might inform clinical risk stratification and treatment strategies**
- **Need for methods to reconstruct tumor evolutions maps, by exploring its genomic landscape**

2. Aims of the project

Reconstruct the clonal evolution trajectories in GBM to identify:

1. Recurrent patterns of complex structural variants (cSVs) during tumor evolution
2. Mechanisms of treatment resistance during progression

3. Material and methods

Whole Genome Sequencing (WGS) data of paired samples from glioblastoma patients

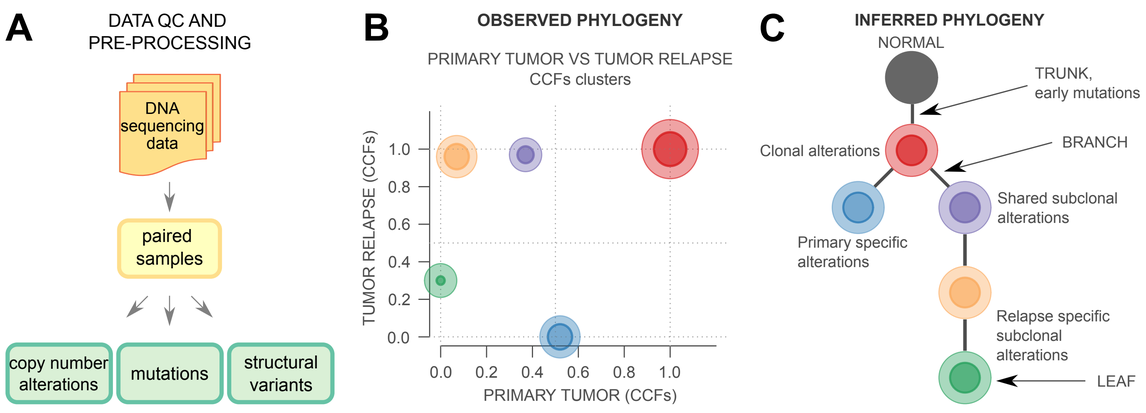


Figure 1 - Clonal evolution reconstruction workflow.
A) Quality control (QC) and data pre-processing. **B)** The cancer cell fractions (CCFs, defined as the fraction of cancer cells at the given state) clustering informs the subclonal reconstruction. **C)** The clone tree is represented as a truncal node giving rise to different selected subclones within it.

4. Results

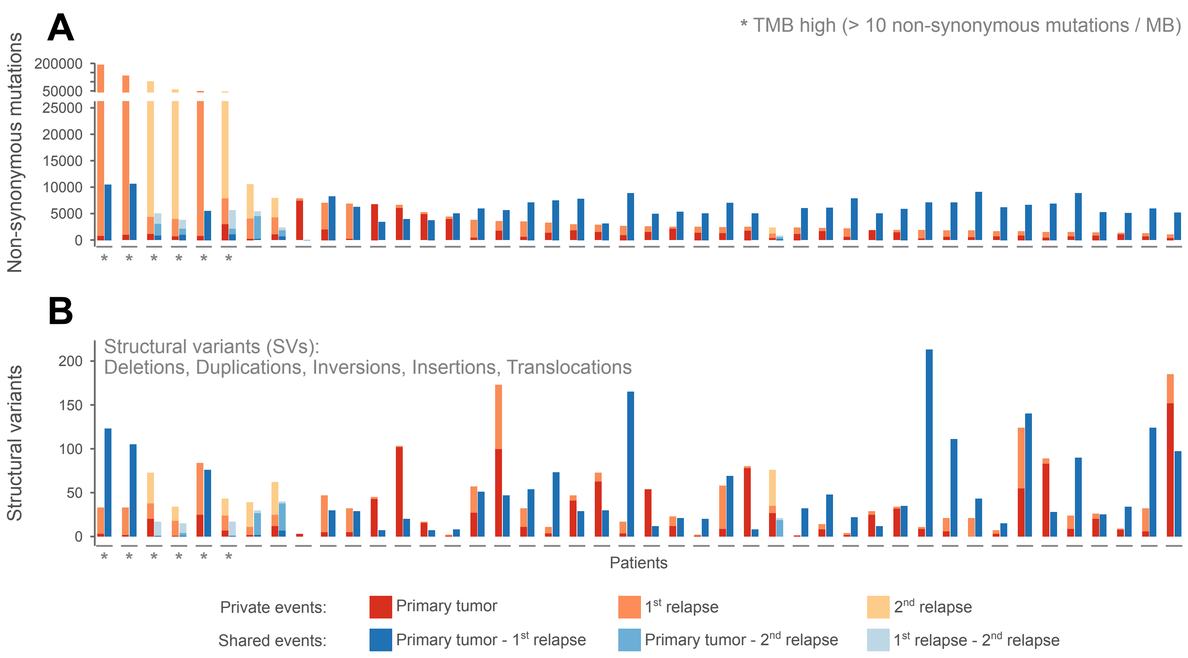


Figure 2 - Hypermutation and low clonal divergence separate GBM patients.
Number of non-synonymous mutations (**A**) and structural variants (**B**) across different time-points, grouped by patient. Patients with high number of private events exhibit high clonal divergence, whereas patient characterized by high number of shared events exhibit lower clonal divergence.

5. Future perspectives

- Integrate the subclonal reconstruction with treatment history;
- Identify recurrent patterns and mechanisms of ecDNA evolution;
- Examine recurrent patterns of mutations and complex SVs;
- Explore the etiology of complex SVs and look for correlations with treatment history

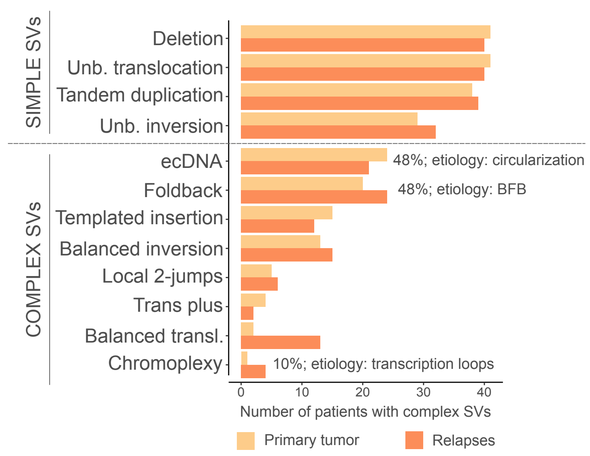
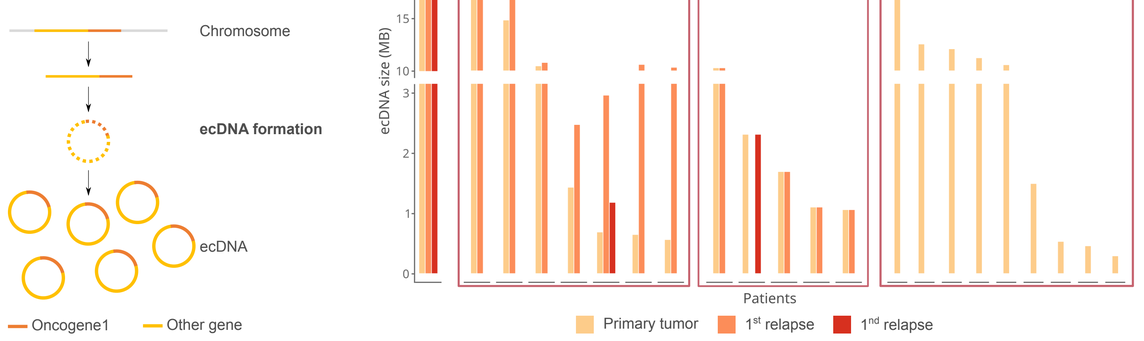


Figure 3 - Most SVs are preserved between the time-points
The most common complex SVs are ecDNA and Foldback.

ecDNA:
- Set of genomic intervals connected together in a circular ("plasmid-like") structure and amplified in terms of copy number
- Often carries one or more oncogenes
- Non-mendelian segregation

Figure 4 - ecDNA follows different evolutionary trajectories



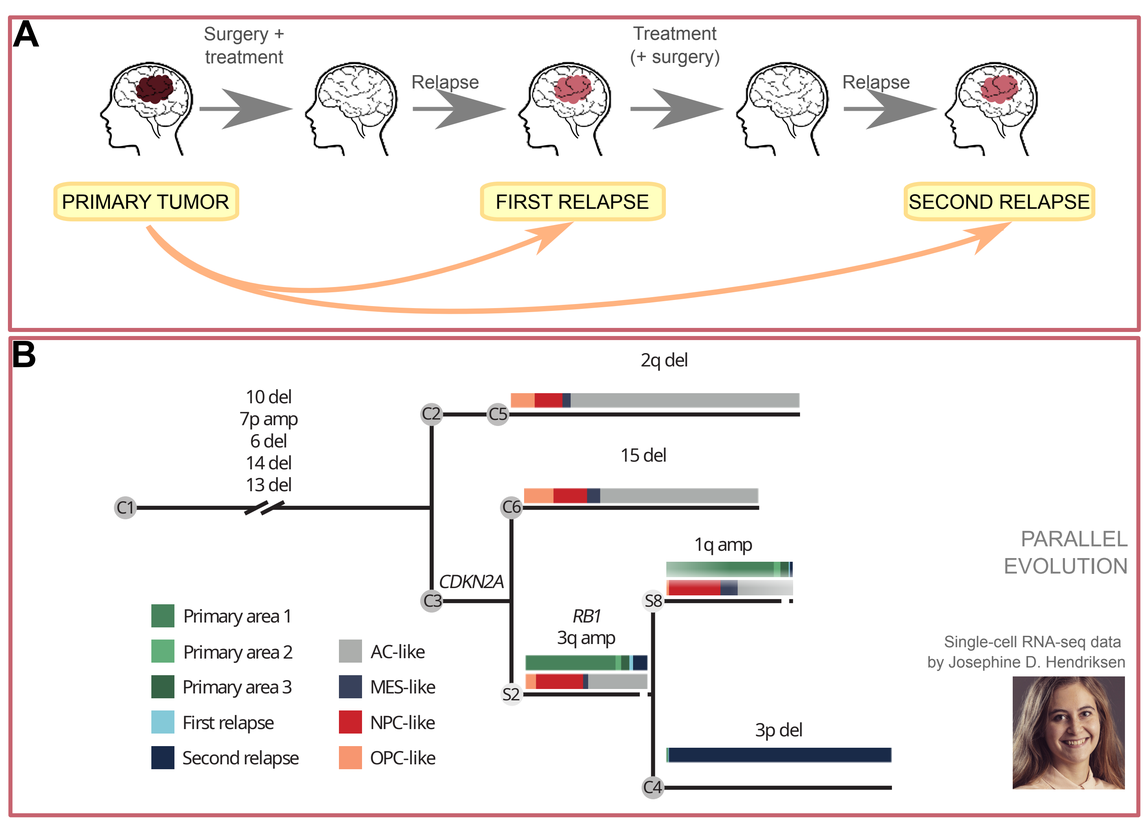


Figure 5 - Example of parallel evolution. Integrating the clonal evolution reconstruction with single-cell sequencing data allows to uncover small clones which are undetectable through WGS data only.
A. Sketch representing the surgery timeline. Orange arrows highlight the branched evolution trajectory. **B)** Phylogenetic tree of tumour subclones identified through WGS and scRNA-seq. Barplots along each branch display the tissue composition (top) and tumour cell states (bottom) present in the corresponding subclone.

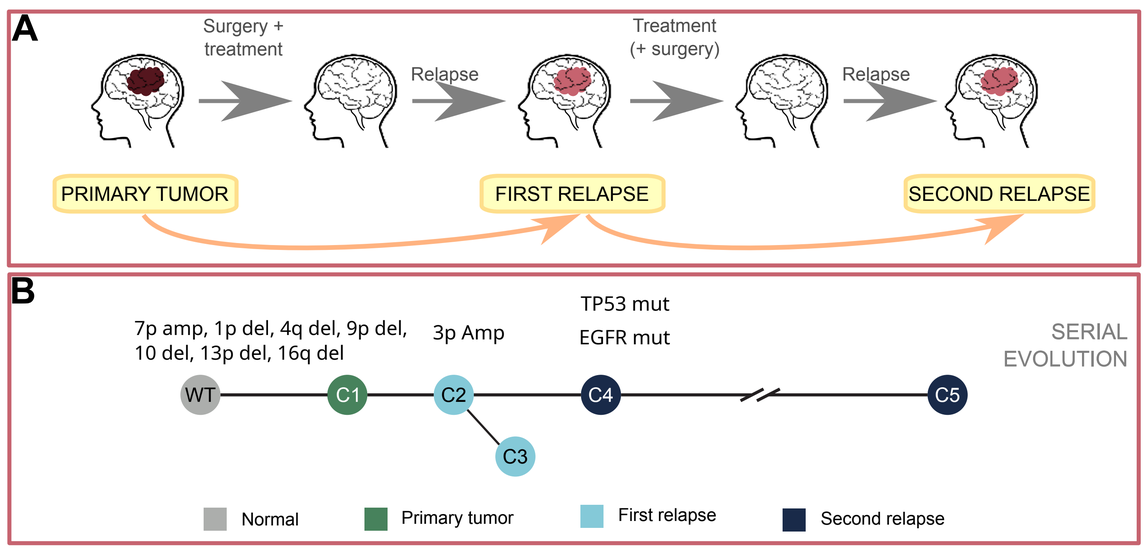


Figure 6 - Example of serial evolution trajectory.
A) Sketch representing the surgery timeline. Orange arrows highlight the linear evolution trajectory. **B)** Phylogenetic tree of tumour subclones identified trhough WGS only.