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STUDY OF INTRA-TUMOUR GENOMIC HETEROGENEITY AND ACQUIRED RESISTANCE TO TYROSINE KINASE INHIBITORS IN LUNG CANCER

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Introduction

Patient with lung cancer with **EGFR, MET, or RET alterations** initially respond to Tyrosine Kinase Inhibitor's (TKIs), but resistance inevitably develops through diverse mechanisms. Evidence suggests that **resistant subclones may pre-exist before treatment and expand under drug pressure**. Using **single-cell DNA and RNA sequencing**, we characterized **intratumoral heterogeneity** in resistance models to understand how cellular diversity drives treatment failure and to guide future combination strategies.

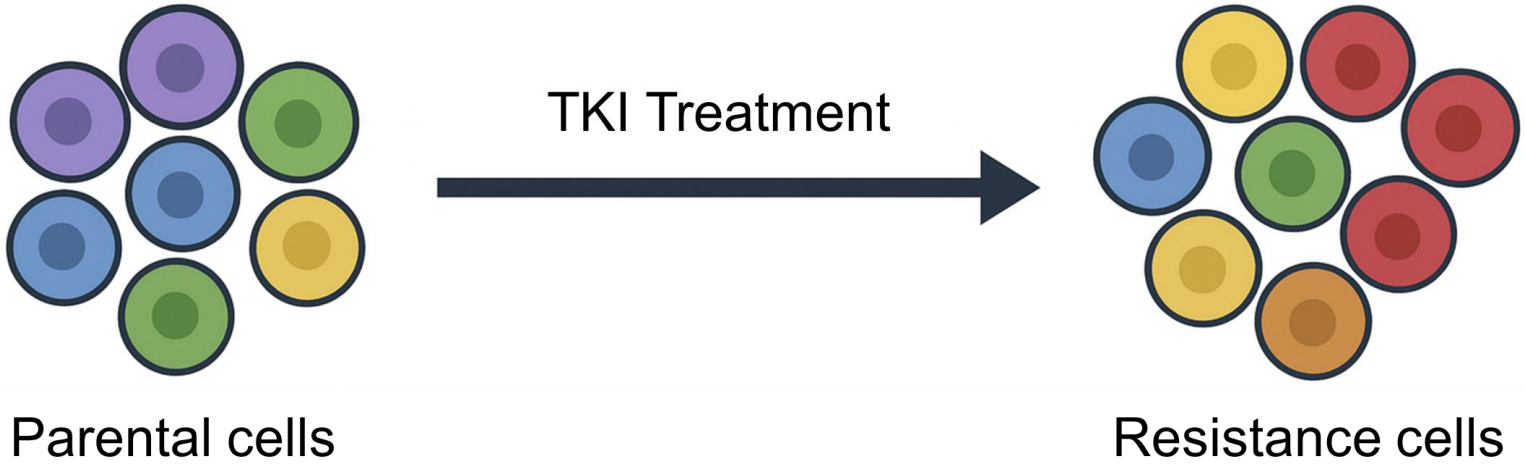

Figure 1. Conceptual model: minor resistant clones pre-exist and expand under TKI treatment, driving relapse.

Cell Models

1) **EGFR Models** (A):
- PDC5 (Parental)
- PDC5.2 (In vivo Resistant) (Pros et al. Ann Oncol 2020)
- PDC5-AR5 (In Vitro resistant)

2) **MET Models** (B):
- EBC1
- EBC1-PR4 (Gimenez-Xavier et al Mol Cancer Ther. 2017)

3) **RET Models** (C and D):
- PDC6 (BR&LR)
- LC2AD (BR&LR)

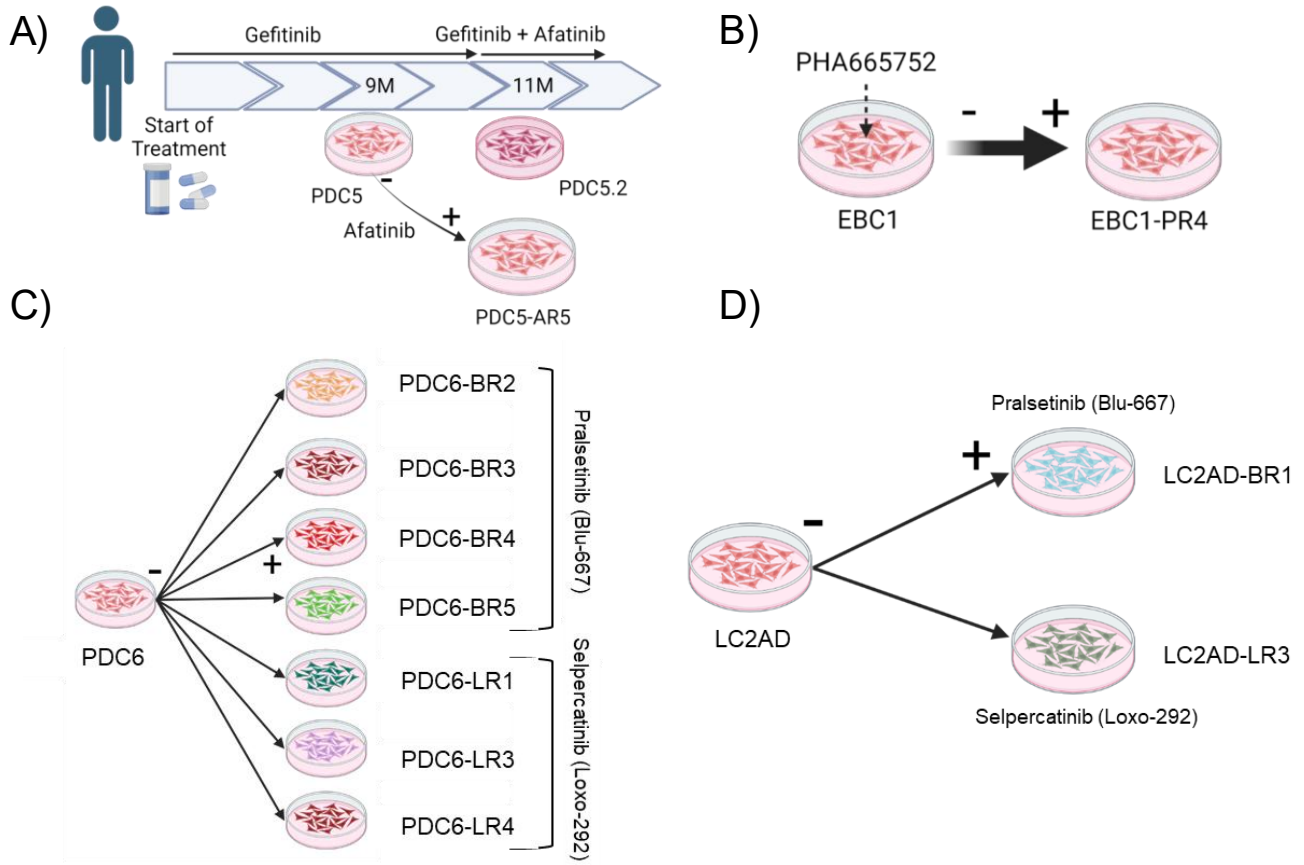

Figure 2. Cell lines models origin.
(A) Timeline of patient-derived cells (PDC): parental PDC5, resistant PDC5.2 (patient-acquired), and in vitro–derived resistant PDC5-AR5. (B) Generation of resistant cells in vitro: parental EBC1 and resistant EBC1-PR4. (C) RET-patient derived cells model (PDC6): derivation of multiple resistant clones (PDC6-BR and PDC6-LR) from parental PDC6. (D) RET-cell line model (LC2AD): generation of resistant lines LC2AD-BR1 (Blu-667) and LC2AD-LR3 (Loxo-292).

Experimental Workflow

1) Patient-derived cancer cells (PDCs) and lung cancer cells commertially avaialble and their respective resistant derivative cells used in the study.

3) Single-cell sequencing (scDNA-seq and scRNA-seq) to profile genomic and transcriptomic heterogeneity.

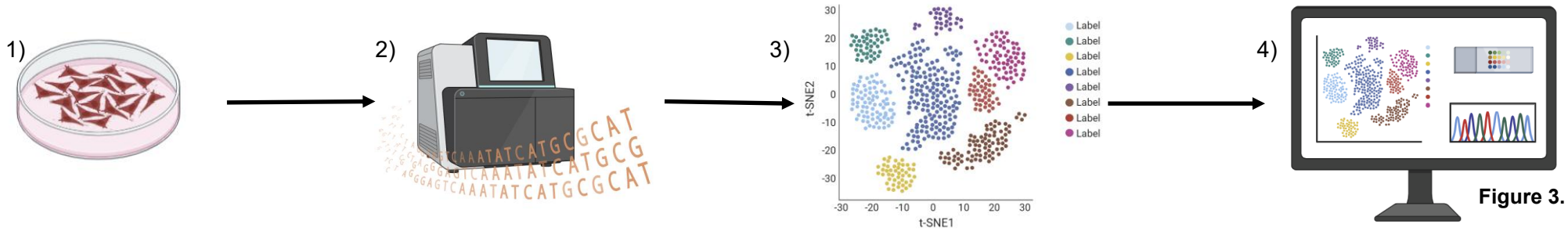

Figure 3. Experimental workflow.

2) Bulk next-generation sequencing (RNA-seq and WES) were used to identify genomic alterations arising after acqurired resistance and to design scDNA panels.(

4) Bioinformatic analysis

Bulk Data Analysis

WES results

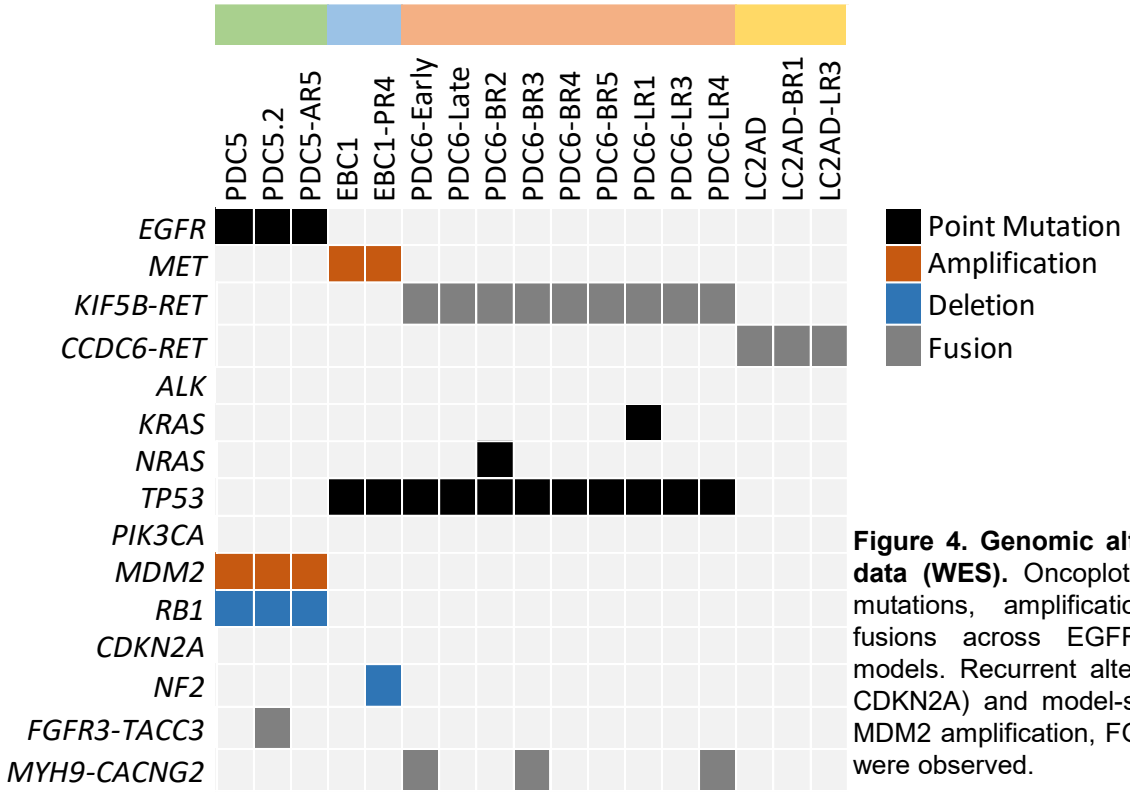

Figure 4. Genomic alterations from bulk data (WES). Oncoplot summarizing driver mutations, amplifications, deletions and fusions across EGFR, MET and RET models. Recurrent alterations (TP53, RB1, CDKN2A) and model-specific events (e.g., MDM2 amplification, FGFR3-TACC3 fusion) were observed.

RNA-seq Results

- Gene expression profiling allowed the derivation of **resistance-associated signatures**, providing complementary functional insights.
- These **bulk-derived signatures** were subsequently tested in the **scRNA-seq datasets** to explore their distribution across cellular subpopulations.

scRNA-seq reveals heterogeneity in terms of gene expression within the resistant cell populations

In **PDC5** models (~7,900 cells), single-cell analysis revealed **modest intratumoral heterogeneity**, with two subpopulations detectable before treatment and showing similar transcriptional profiles between parental and resistant lines.

In **EBC1** models (~10,200 cells), the analysis indicated a **more homogeneous profile**, with no relevant transcriptional changes between parental and resistant conditions.

- **PDC5:** small pre-existing subpopulations with similar expression profiles.
- **EBC1:** stable transcriptional programs between parental and resistant cells.

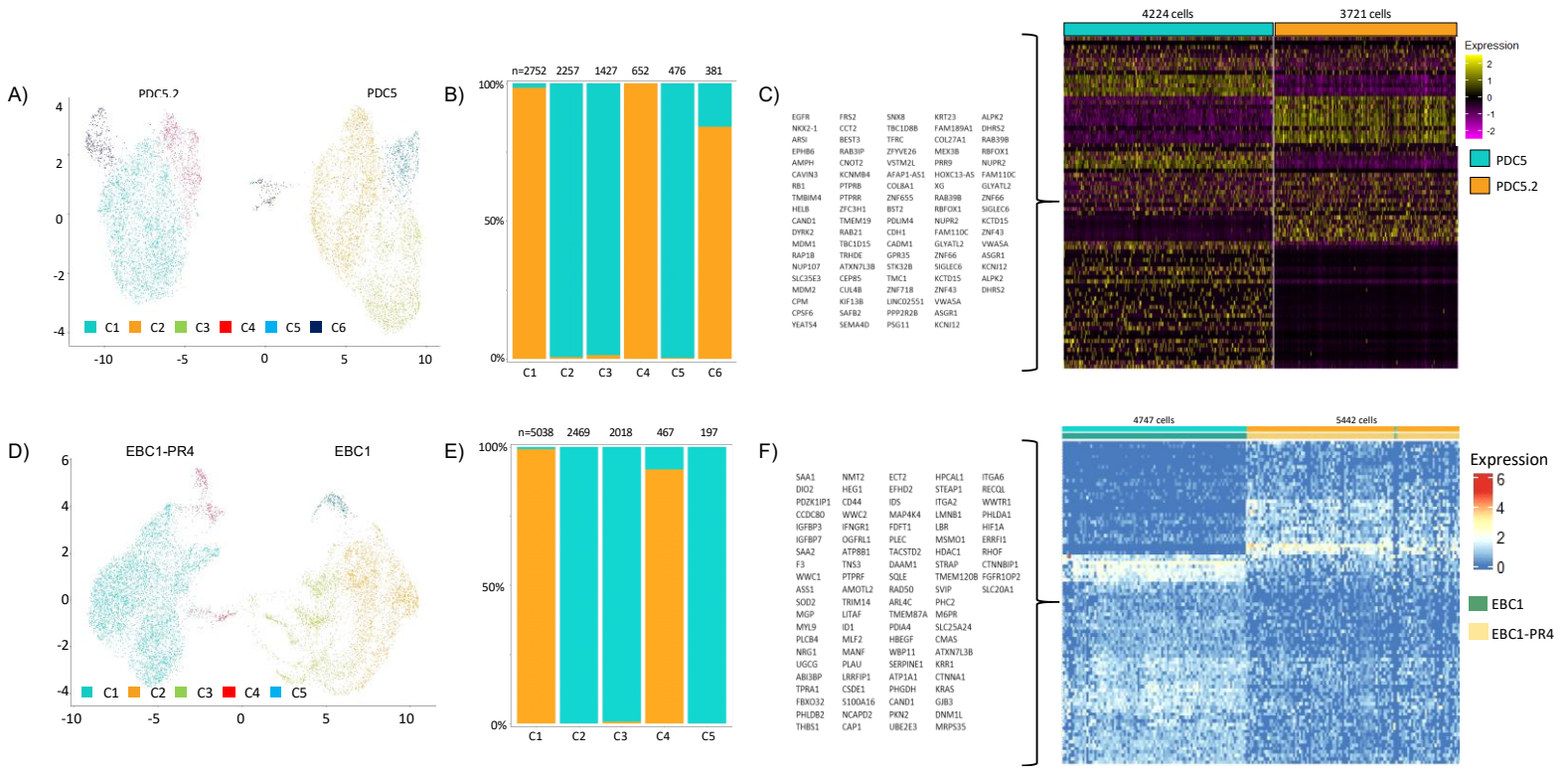

Figure 5. scRNA-seq analysis of PDC5 and EBC1 models. (A,D) UMAP plots reveal distinct cellular subpopulations in parental and resistant cells. (B,E) Barplots show changes in cluster composition, with expansion of specific subsets under TKI pressure. (C,F) Heatmaps illustrate differential gene expression across clusters, validating bulk-derived resistance signatures at the single-cell level.

scDNA-seq: PDC5 models

scDNA analysis revealed consistent **MDM2 amplification** across all EGFR-resistant models (PDC5, PDC5.2, PDC5-AR5). In **PDC5.2**, two distinct resistant subclones were identified: one with focal MDM2 amplification and another with a broader amplification that also included the **FGFR3-TACC3 fusion**. **UMAP projections and barplots separated these subclones and showed shifts in their proportions**, while CNV heatmaps highlighted stronger MDM2 amplification in PDC5.2 compared with the other models.

- **All EGFR models:** MDM2 amplification detected.
- **PDC5.2:** two resistant subclones (focal MDM2 amp vs. broad amp + FGFR3-TACC3 fusion).

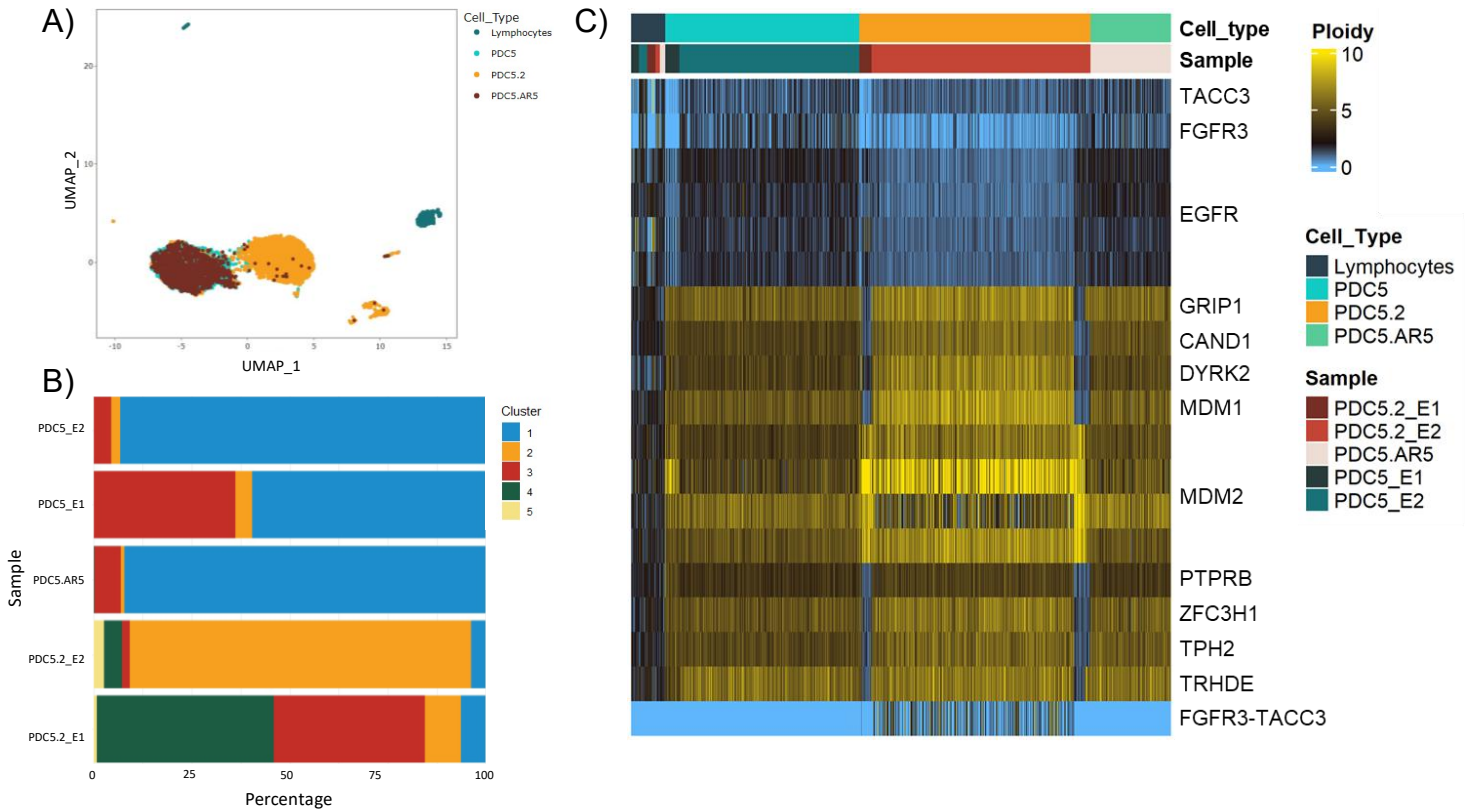

Figure 6. scDNA-seq analysis of EGFR-driven models. UMAPs, clonal barplots, and CNV heatmaps show MDM2 amplification in all models. PDC5.2 displays two subclones: one with focal MDM2 amplification, and another with non-focal amplification including FGFR3-TACC3 fusion.

scDNA-seq: EBC1 models

- Single-cell DNA analysis revealed clear divergence between **parental** and **resistant (PR4)** cells. **UMAP projections and barplots** showed separation of subclones and shifts in clonal composition under TKI pressure. **CNV heatmaps** confirmed **NF2 loss** and increased **MET copy number** in resistant cells, indicating clonal selection and reinforcement of MET signaling as resistance mechanisms.
- Findings indicate **clonal selection** and **reinforcement of MET signaling** as resistance mechanisms.

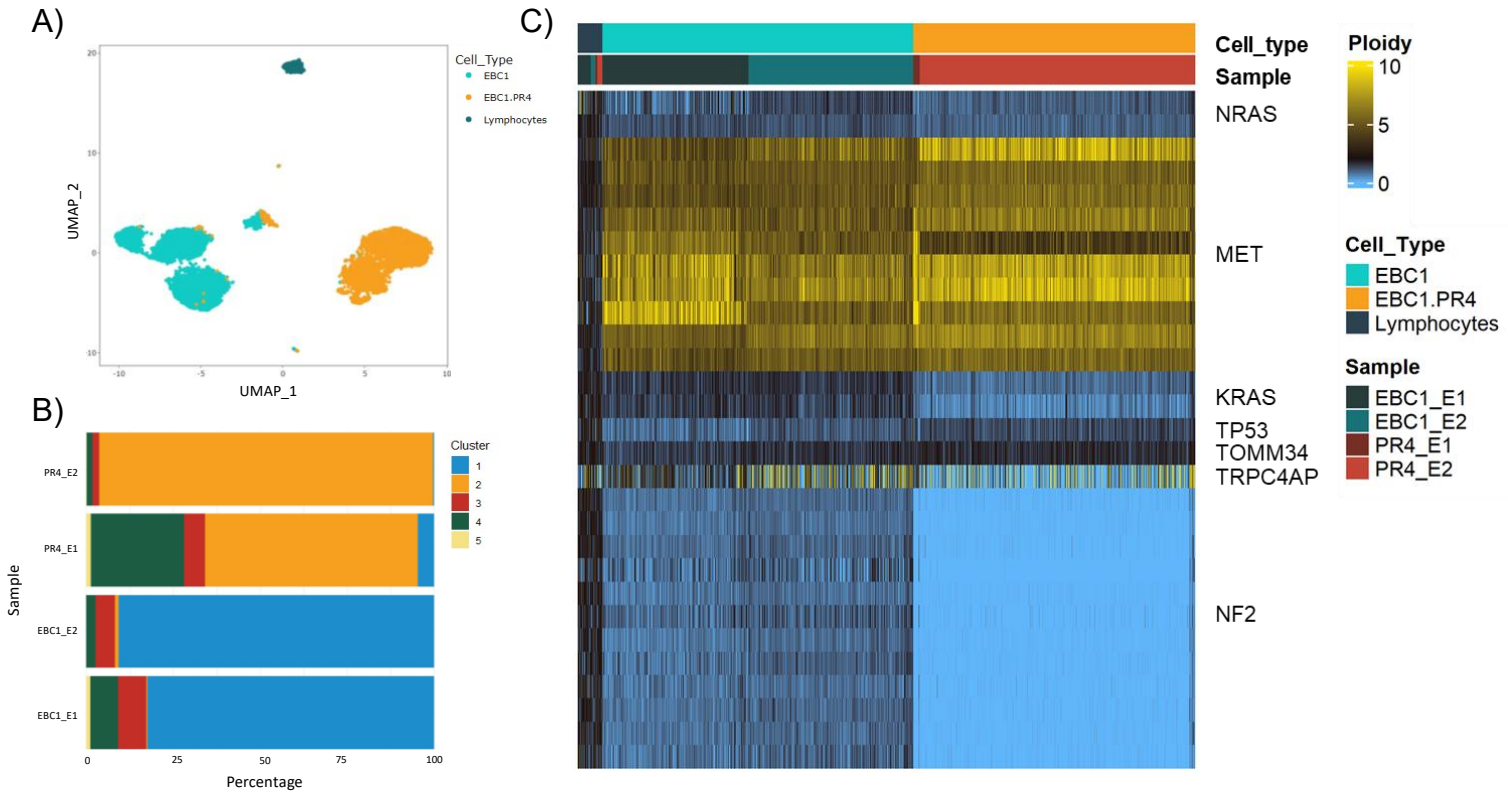

Figure 7. scDNA-seq analysis of the MET-driven EBC1 model. (A) UMAP projection shows distinct clustering of parental and resistant cells. (B) Barplot illustrates changes in clonal composition after TKI treatment. (C) CNV heatmap highlights NF2 loss and an increased MET copy number gain in resistant PR4 cells compared to parental populations..

scDNA-seq: PDC6 models

Single-cell DNA analysis revealed **clonal heterogeneity** across resistant lines. **Barplots** showed expansion of resistant-specific clusters, while the **UpSet plot** highlighted both unique and shared mutations. **CNV heatmaps** identified a **MYH9–CACNG2 fusion**, **RET copy gain**, and **PIK3CA LOH**, indicating resistance driven by a mix of line-specific and convergent events.

- **Barplots:** expansion of resistant clusters.
- **UpSet plot:** unique + shared mutations across lines.
- **CNV heatmap:** MYH9–CACNG2 fusion, RET gain, PIK3CA LOH.

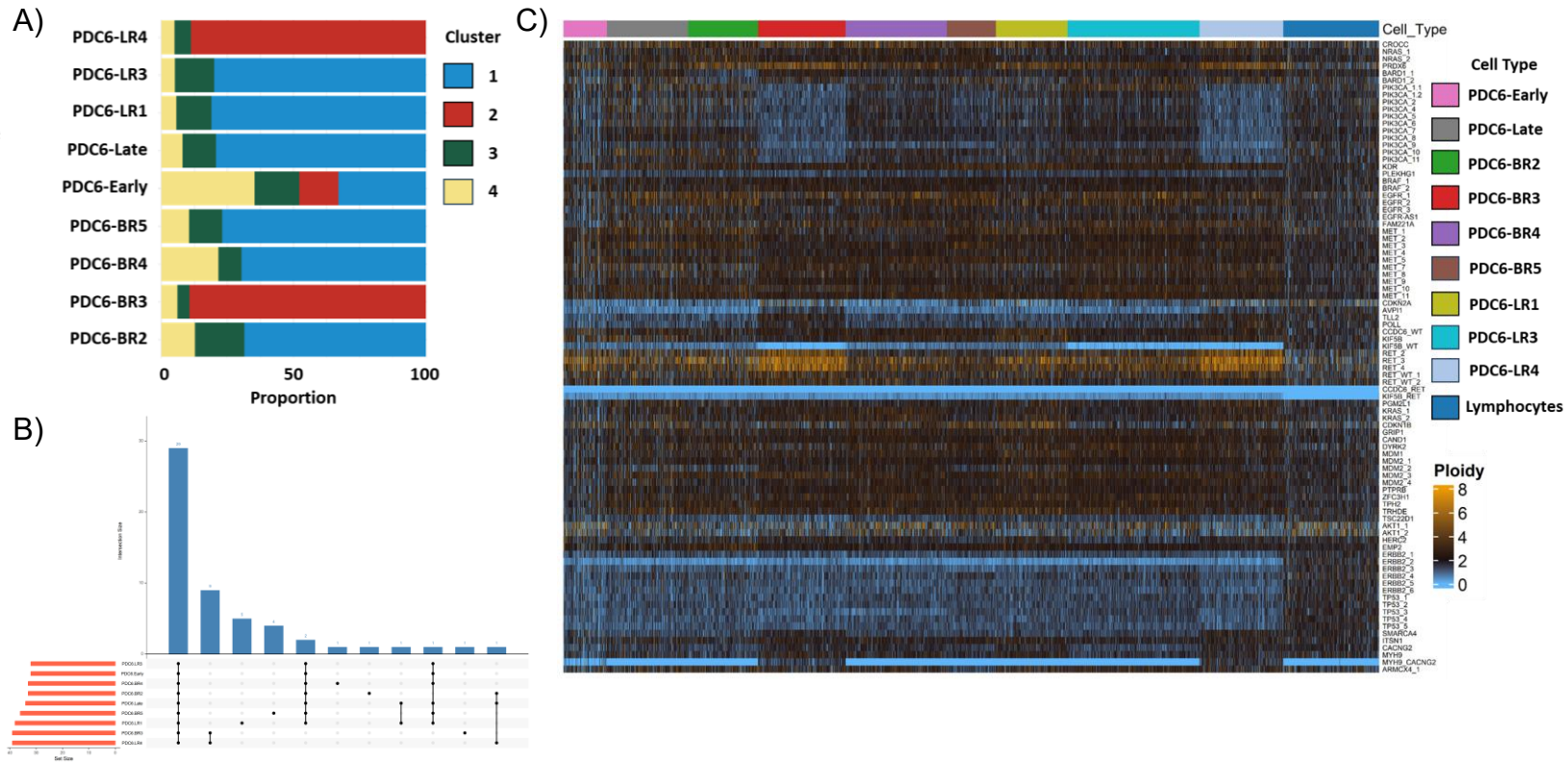

Figure 8. scDNA-seq of PDC6 (RET). (A) Clonal proportions highlight expansion of resistant subclones. (B) UpSet plot shows private mutations and shared events across models. (C) CNV heatmap reveals RET copy-number alterations.

scDNA-seq: LC2AD models

Single-cell DNA analysis revealed **distinct resistant clones (BR1, LR3)** compared to parental cells. **Barplots** showed clonal shifts with expansion of resistant-specific populations, while the **Venn diagram** highlighted both unique mutations and a shared core of alterations. **CNV heatmaps** confirmed **PIK3CA loss in both clones** and a stronger **AKT copy gain in LR3**, indicating resistance driven by shared vulnerabilities and clone-specific adaptations.

- **Barplots:** resistant clones expanded vs. parental.
- **Venn diagram:** unique and shared mutations across BR1/LR3.
- **CNV heatmap:** PIK3CA LOH (both); AKT gain (LR3-specific).

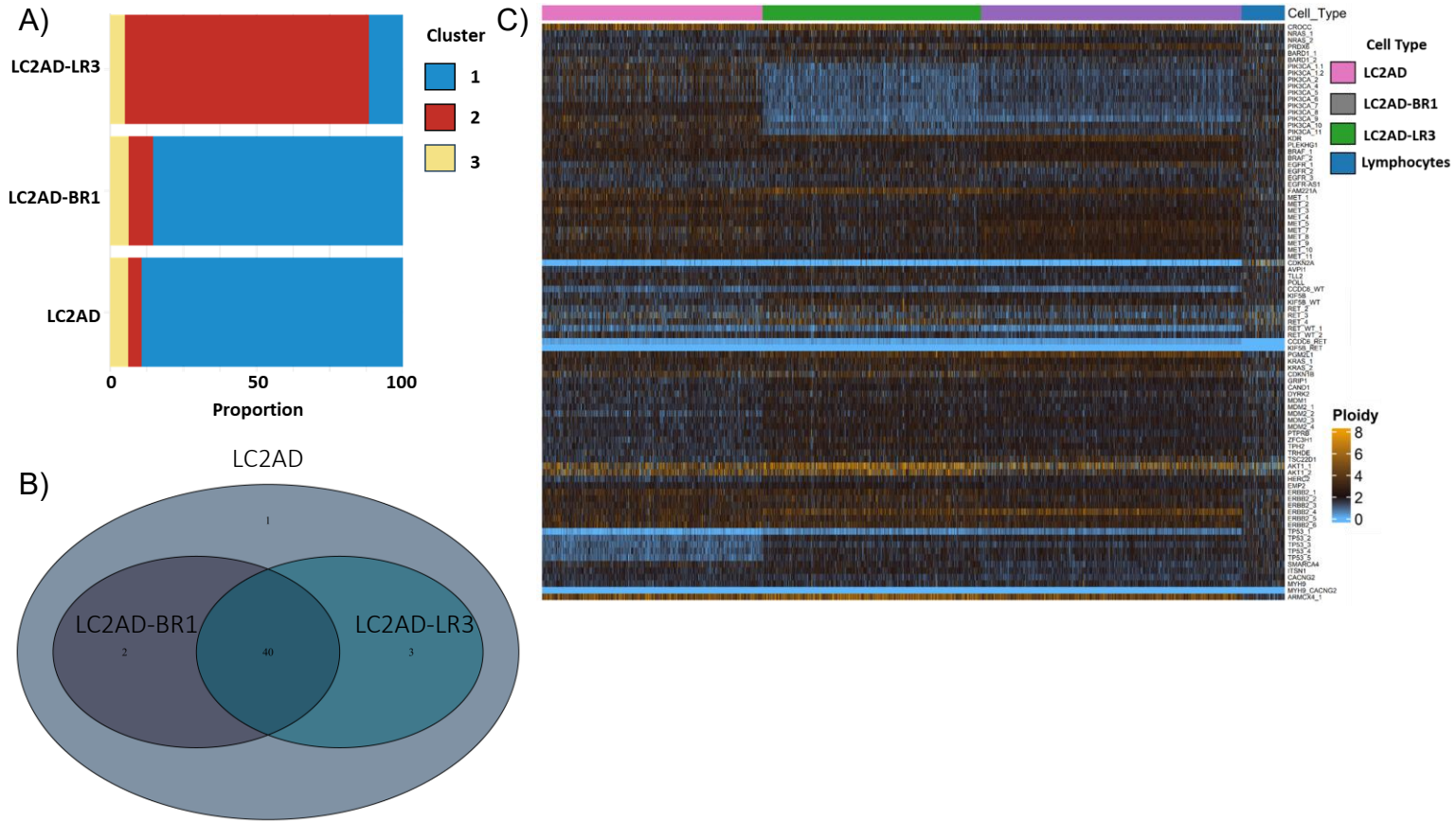

Figure 9. scDNA-seq of LC2AD (RET). (A) Clonal proportions highlight expansion of resistant subclones. (B) Venn diagram shows private mutations and shared events across models. (C) CNV heatmap reveals RET copy-number alterations.

Conclusions

- **Bulk and single-cell sequencing (DNA/RNA)** revealed extensive clonal heterogeneity across EGFR-, MET-, and RET-driven lung cancer models.
- **MDM2 amplification** was recurrent across models, with **PDC5.2 (EGFR)** showing distinct resistant subclones: one with **focal amplification** and another with **non-focal amplification** linked to the **FGFR3–TACC3 fusion**.
- **EBC1 (MET model)** resistant clones displayed **NF2 loss** and **increased MET copy number gain**, supporting reinforcement of the MET pathway as a resistance mechanism.
- **RET models (PDC6 and LC2AD)** revealed both **line-specific mutations** and **shared alterations**, including **MYH9–CACNG2 fusion**, **PIK3CA LOH**, and **AKT copy number gain** in specific resistant clones.
- Resistance arises through a combination of **clonal selection of pre-existing populations** and **de novo genomic events**, with multiple mechanisms coexisting within the same model.
- **Key message:**
  - Resistance does not simply “emerge”; in many cases, it is **already present** at low frequency and expands under TKI pressure.
  - **Monitoring subclonal architecture** may help anticipate treatment failure and guide rational combination strategies.

Reference & Acknowledgements

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Thank You

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