Acute myeloid leukemia shows reduced growth rates after chemotherapy

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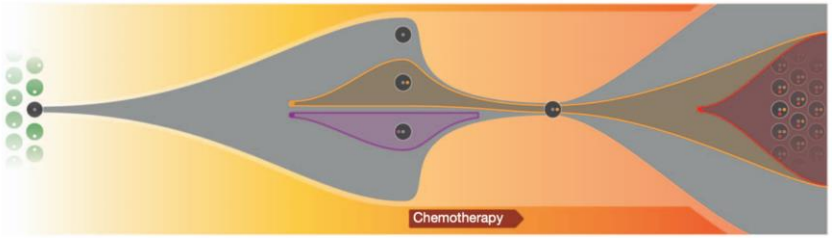
Acute Myeloid Leukemia (AML)

- Abnormal proliferation of cells from the **myeloid lineage** in the bone marrow
- High **relapse** frequency after chemotherapy
- High intratumoral heterogeneity



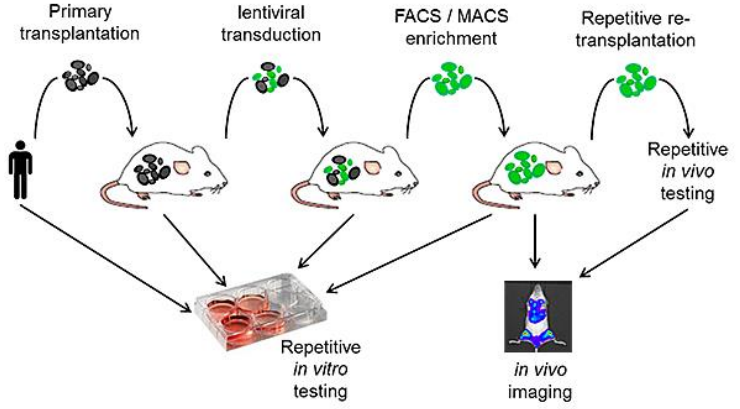
Ding et al. (2012), Nature

Research question

Which molecular changes characterize tumor cells during AML progression and relapse under repeated chemotherapy?

Patient derived xenografts (PDX)

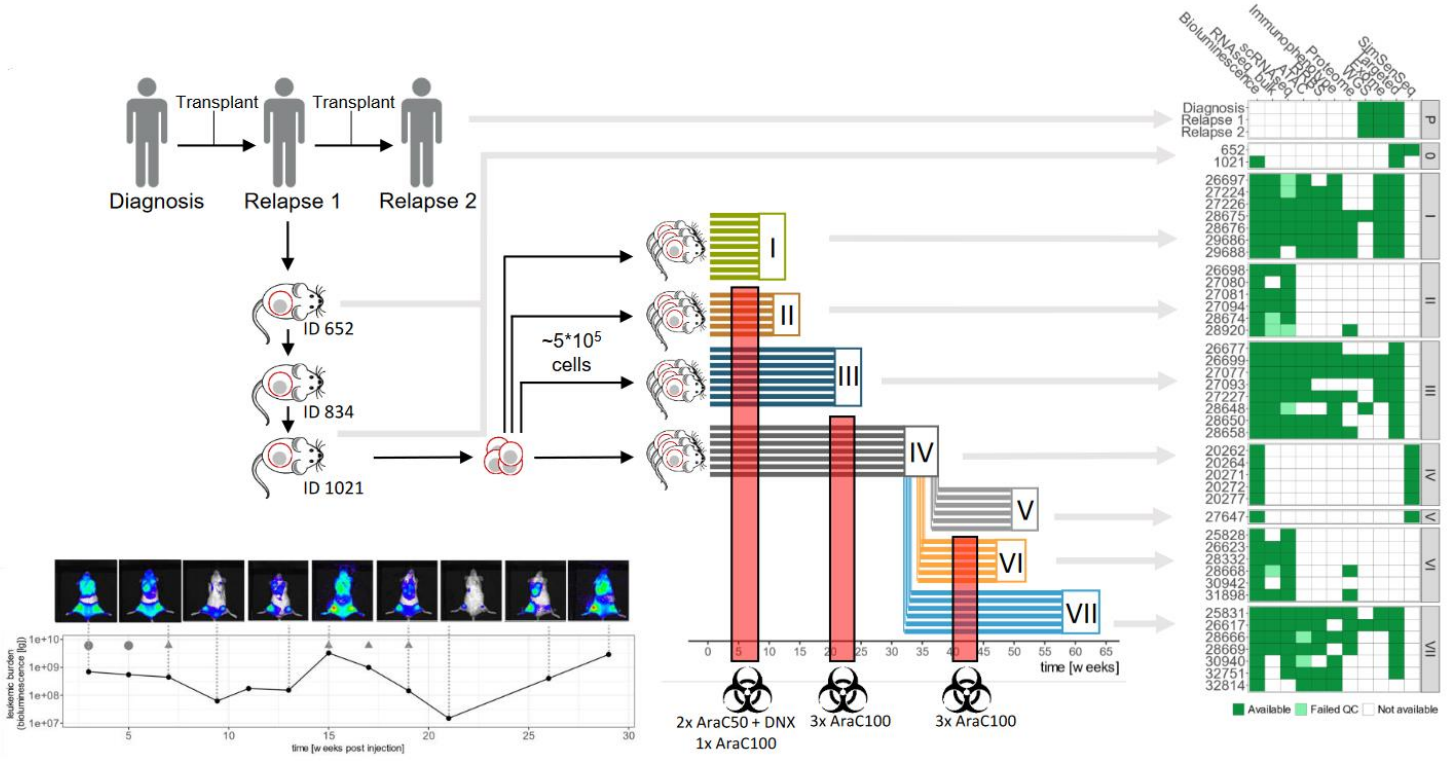
- Engraft primary tumour cells into immuno-deficient mice
- Monitor tumour burden by in vivo imaging



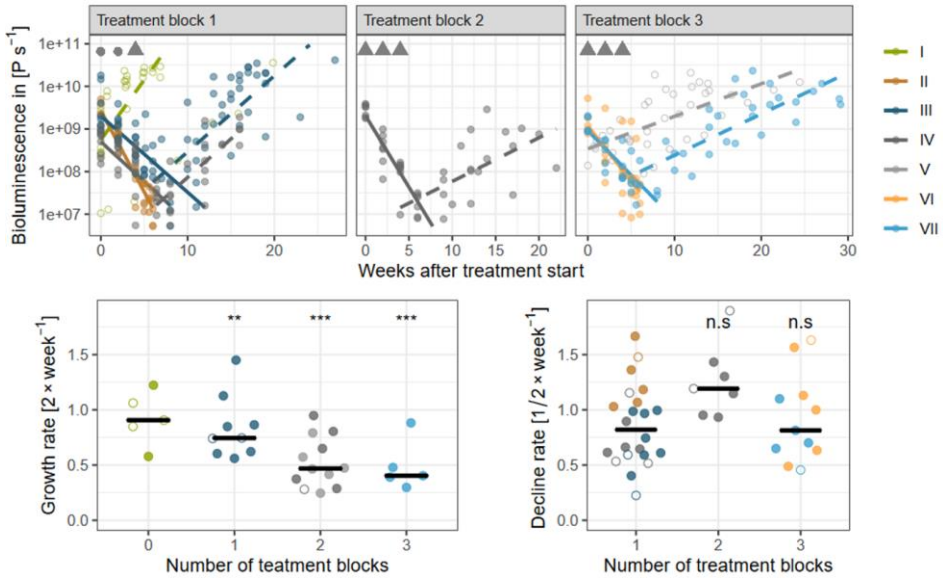
Experimental design

Create AML-PDX model	Repeated chemotherapy treatment	Multi-omics analysis

• Sample from an AML patient after first relapse
• Initial amplification
• Transduction to express firefly luciferase
	• 3 treatment blocks (1: Cytarabine+DNX, 2&3: Cytarabine)
	• 7 treatment groups differing in the
	  ○ Number of treatments received
	  ○ Time of sampling (depleted/outgrown)
		• Genome
		• Transcriptome
		• Epigenome
		• Proteome

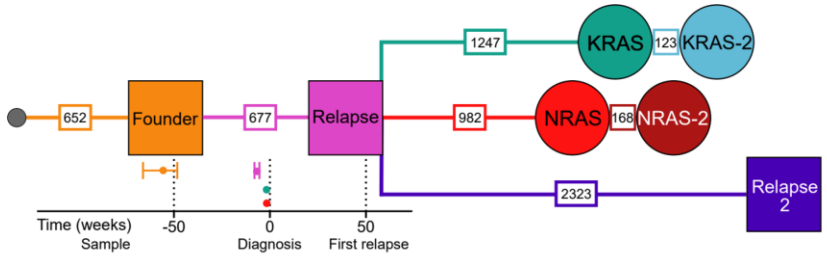


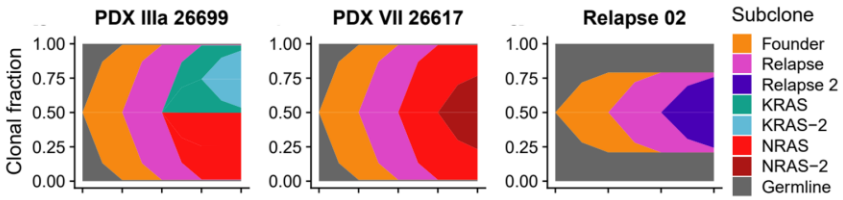
Decline of growth rate after multiple rounds of treatment



- Monitor tumor progression by repeated bioluminescence measurements in PDX mice
- AML-PDX cells did not develop chemo resistance
- AML-PDX cells re-grow **slower** after repeated chemotherapy treatment

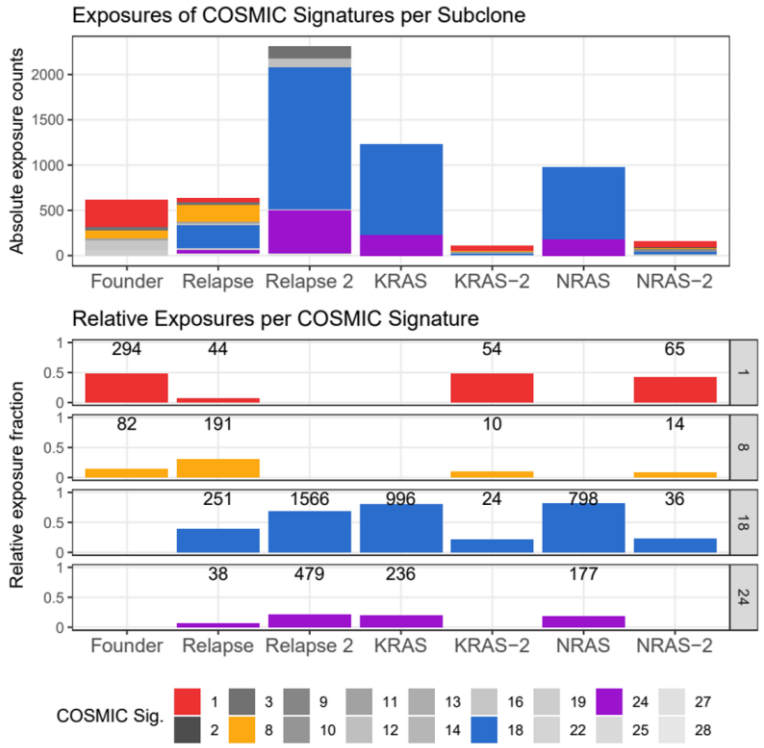
Mutational signature changes after chemotherapy



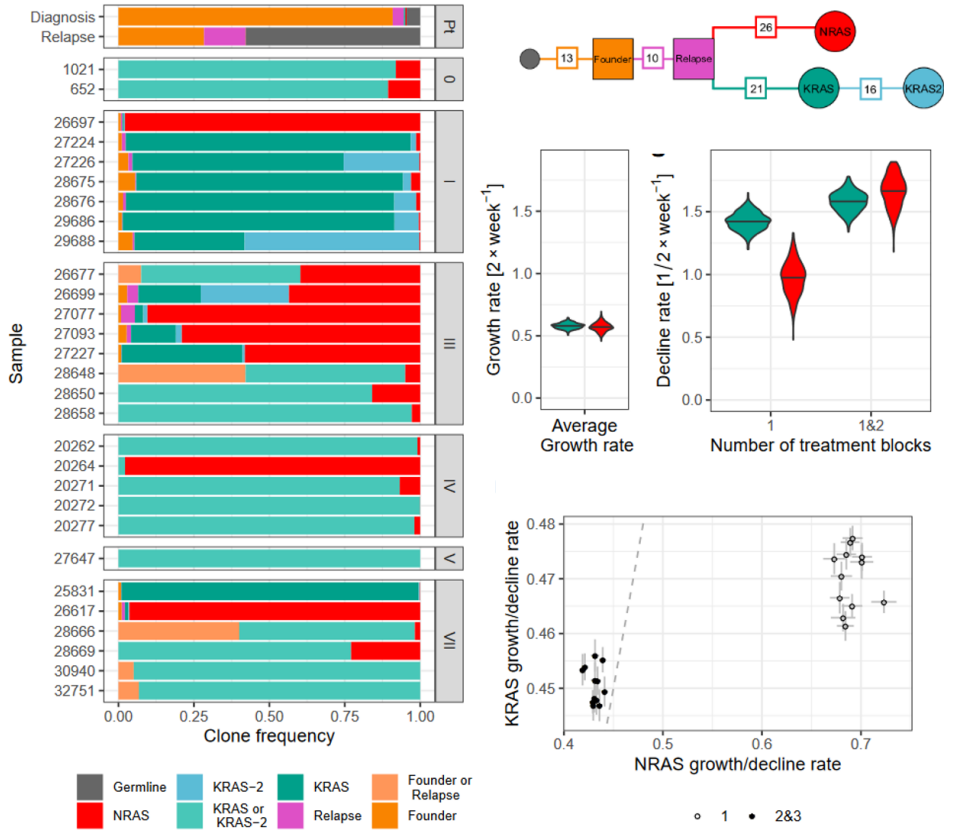


- **Clonal phylogeny** inferred from whole genome sequencing (3 patient samples + 5 PDX-samples)
- Two mutually exclusive subclones (defined by a NRAS or KRAS mutation) become dominant in PDX lines

- Majority of substitutions in NRAS, KRAS and Relapse 2 clone associated with COSMIC **mutational signature SBS18** (reactive oxygen species)

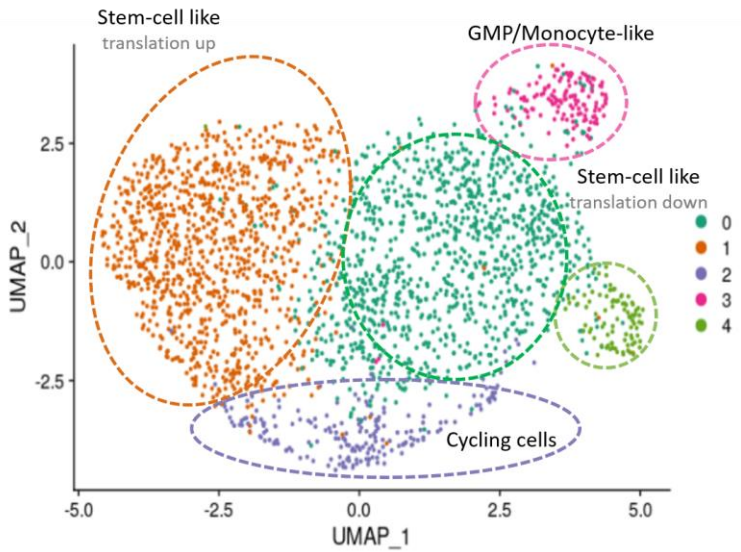


Growth dynamics of subclones

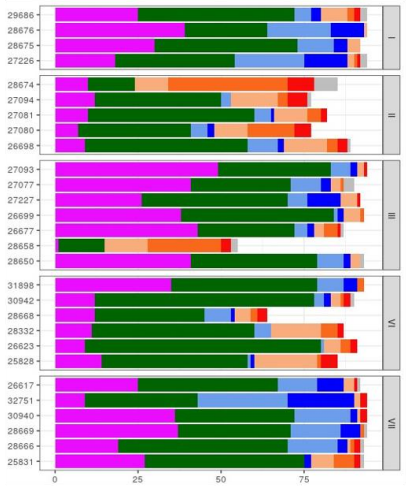


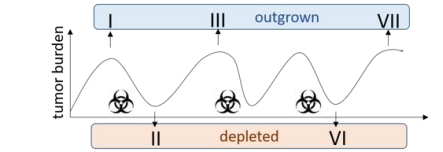
- Whole exome and genome re-sequencing to estimate clone frequencies per PDX sample
- Higher sensitivity of the KRAS clone to the first treatment block (AraC + DNX)

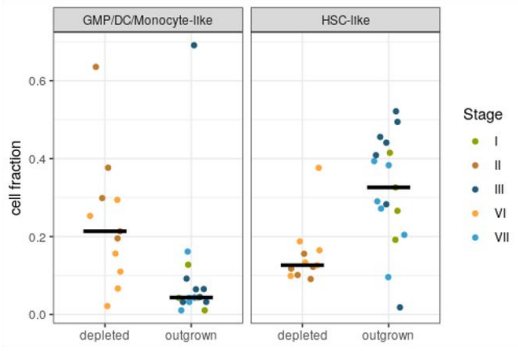
scRNA-seq: changes in the transcriptome and cell composition directly after chemotherapy



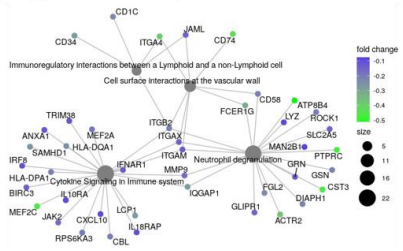
- AML blasts show similarity to different cell types along the myeloid lineage (classification with healthy bone marrow reference)
- Shortly after chemotherapy (**depleted** stages):
  - Higher fraction of more differentiated GMP/monocyte-like cells
  - Upregulation of immune signalling
  - Downregulation of ribosomal and translation associated genes



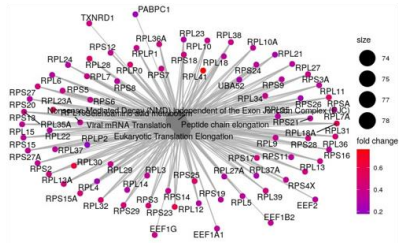




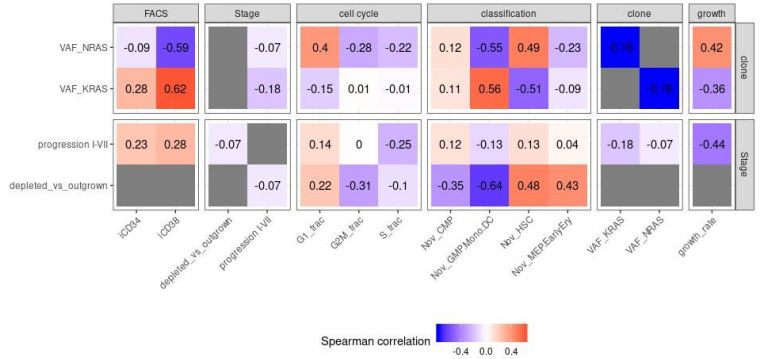
Upregulated in depleted vs untreated



Downregulated in depleted vs untreated



Conclusions



Slow-down in growth rates induced by repeated chemotherapy treatment is associated with a **depletion of stem cell-like features**

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