# Integrated flow cytometry and sequencing to reconstruct evolutionary patterns from dysplasia to acute myeloid leukemia

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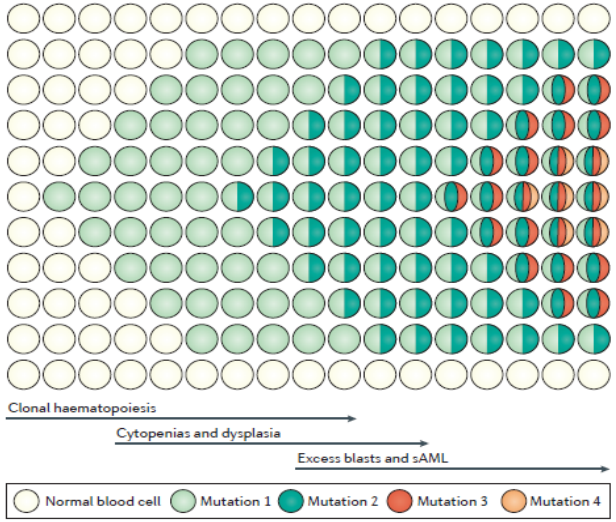
# No disclosures

# Clonal evolution in AML is a highly dynamic process

## And originates long before diagnosis

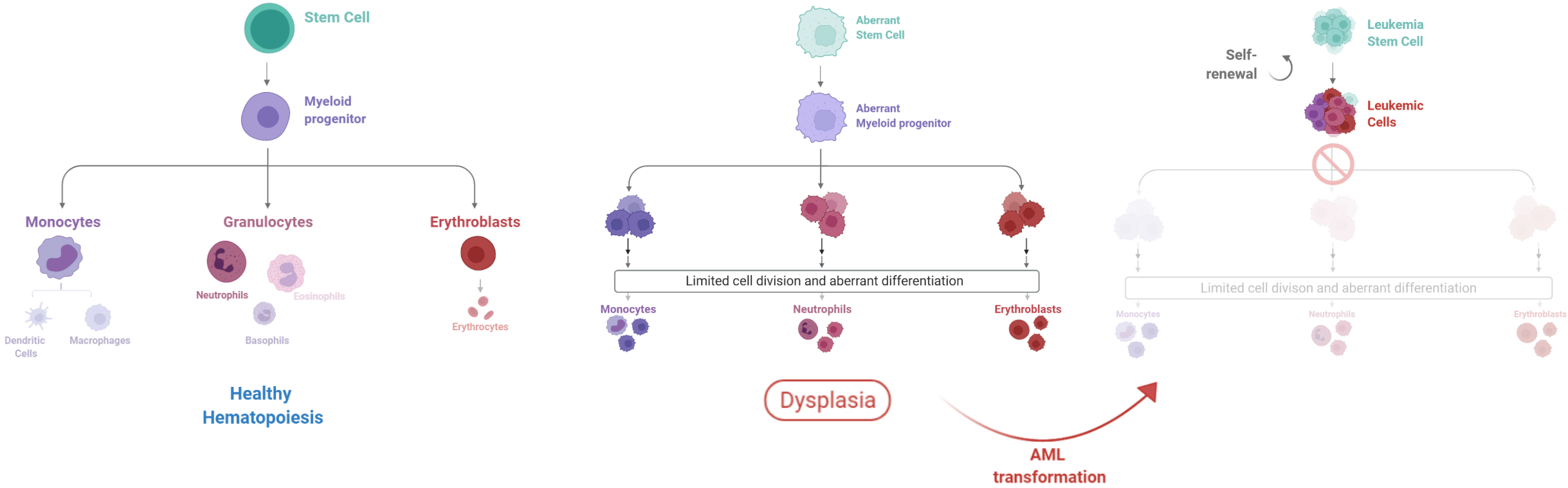
- Having a greater understanding of leukemogenesis may contribute to develop treatment strategies that target the tumor evolutionary process
- However, dissecting leukemic transformation at the onset of AML is challenging without single-cell sequencing
- Most clinical laboratories do not have infrastructure to perform these studies routinely




Sperling A. et al, Nature Reviews Cancer, 2016

# Patients with newly diagnosed AML may present dysplasia

- It could be hypothesized that studying the genetic landscape of dysplastic cells and blasts could uncover the evolutionary process from dysplasia to AML



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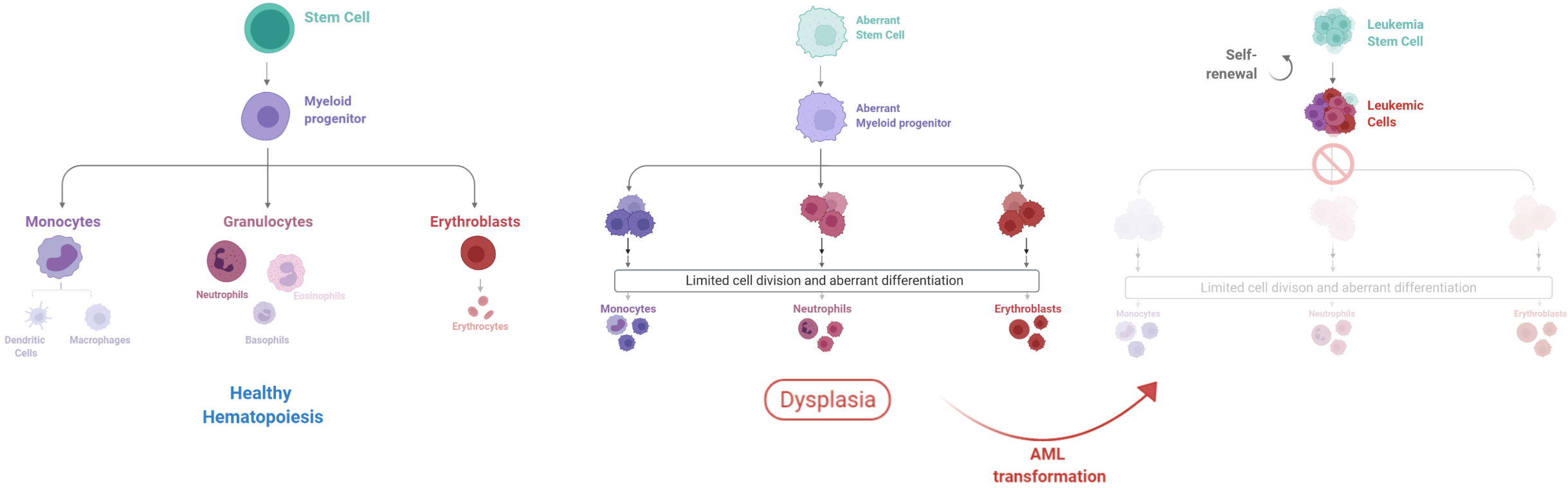



Reconstruct clonal evolution from dysplasia to AML based on the genetic signature of dysplastic cells and leukemic blasts, using MFC and NGS

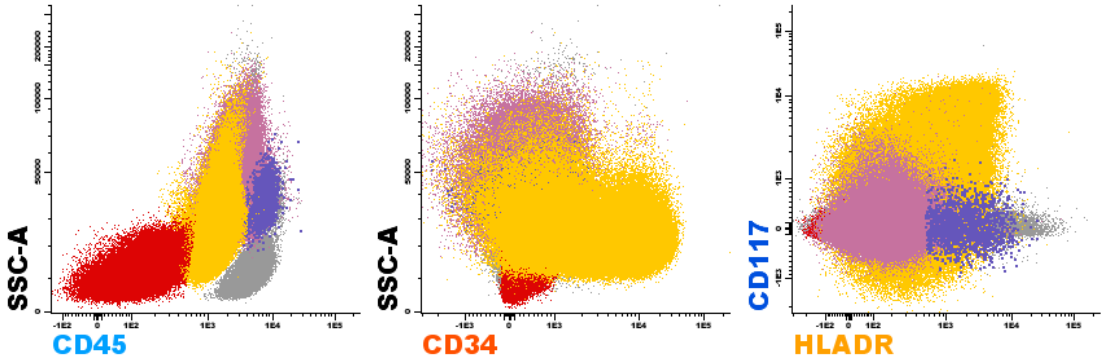
# MFC to detect dysplastic cells and aberrant maturation patterns

## EuroFlow panel for MDS/AML

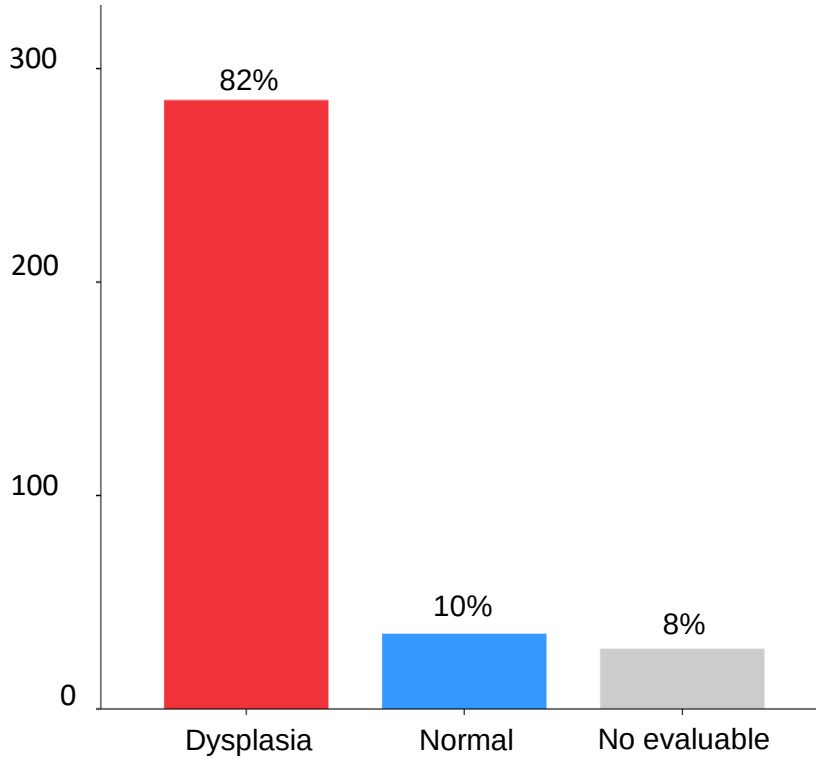
**Diagnosis (N = 348)**

| Tube | Pacific Blue | Pacific Orange | FITC | PE | PerCP-Cy5.5 | PE-Cy7 | APC | APC-H7 | Aim |
|---|---|---|---|---|---|---|---|---|---|
| 1 | HLADR | CD45 | CD16 | CD13 | CD34 | CD117 | CD11b | CD10 | Neutrophil lineage |
| 2 | HLADR | CD45 | CD35 | CD64 | CD34 | CD117 | CD300e | CD14 | Monocytic lineage |
| 3 | HLADR | CD45 | CD36 | CD105 | CD34 | CD117 | CD33 | CD71 | Erythroid lineage |
| 4 | HLADR | CD45 | TdT | CD56 | CD34 | CD117 | CD7 | CD19 | Aberrant |
| 5 | HLADR | CD45 | CD15 | NG2 | CD34 | CD117 | CD22 | CD38 | Aberrant |

- Phenotypes associated with leukemia
- Aberrant maturation patterns

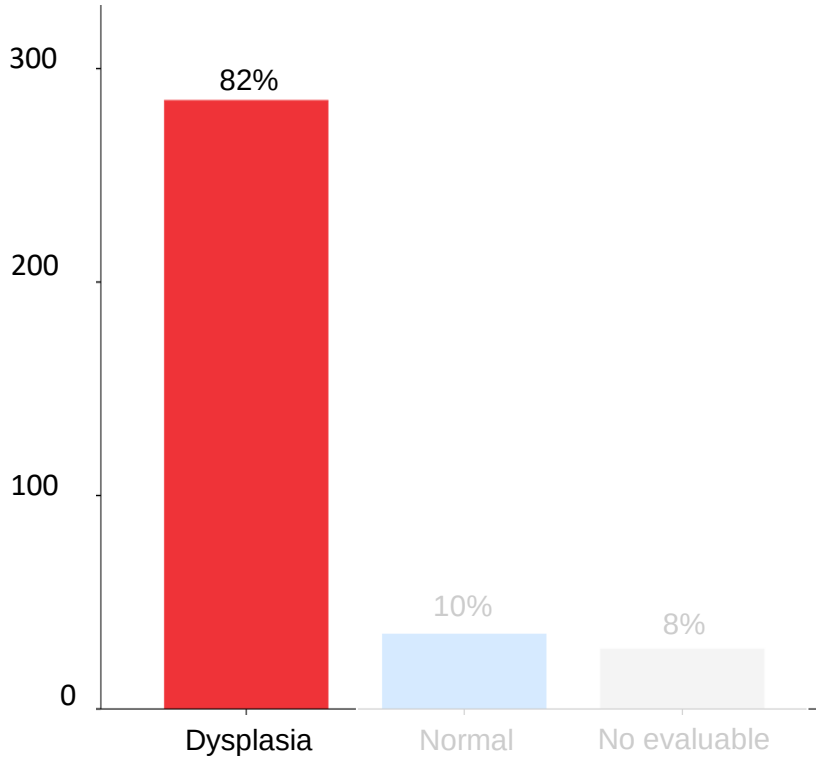


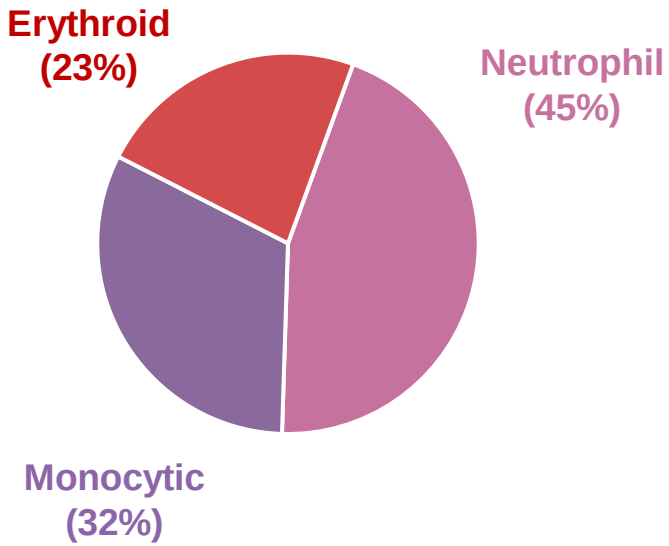
# Dysplastic cells were observed in most patients with newly diagnosed AML, using MFC




- Dysplastic cells were observed in 285 (82%) cases
- Only 35 (10%)  patients showed no signs of dysplasia
- Remaining 28 (8%) cases had undetectable hematopoiesis

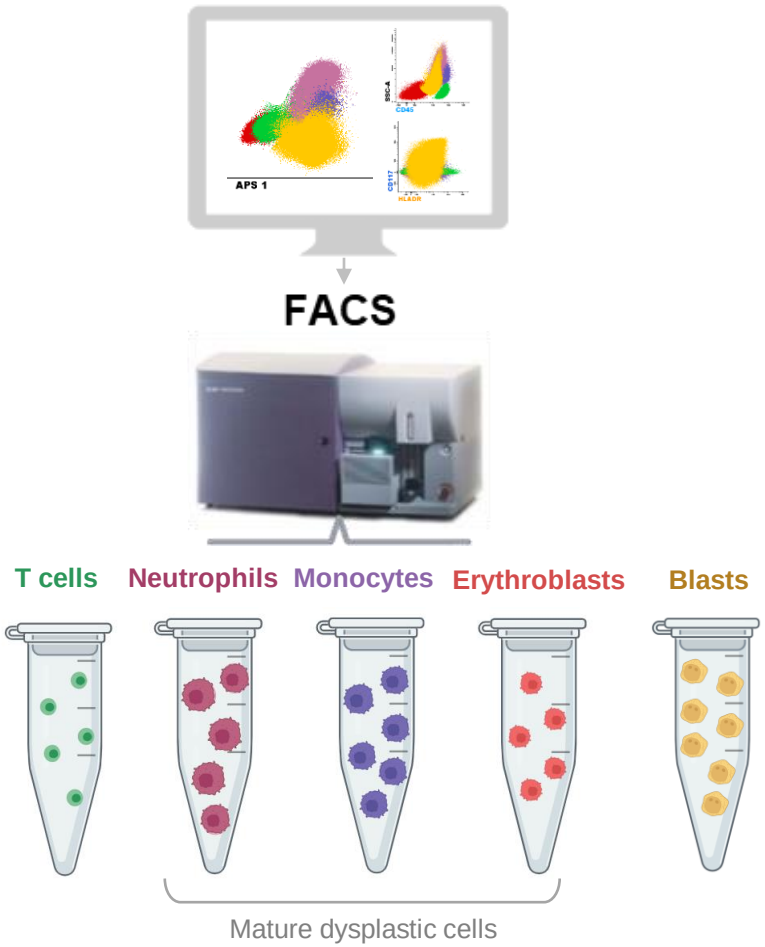
# Dysplasia was most frequent in neutrophils and monocytes




- **53%** multi-lineage dysplasia

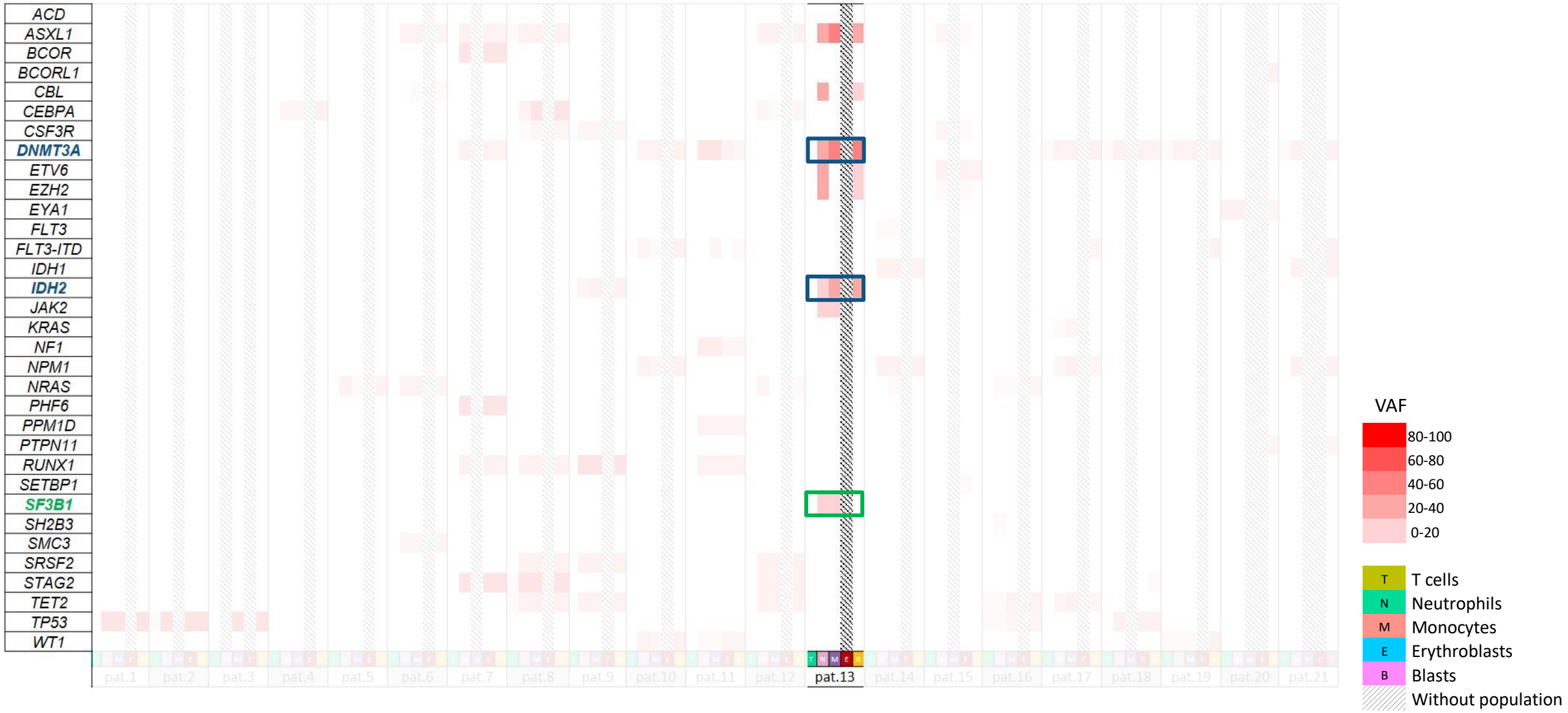
# Genetic characterization of dysplastic cells and blasts



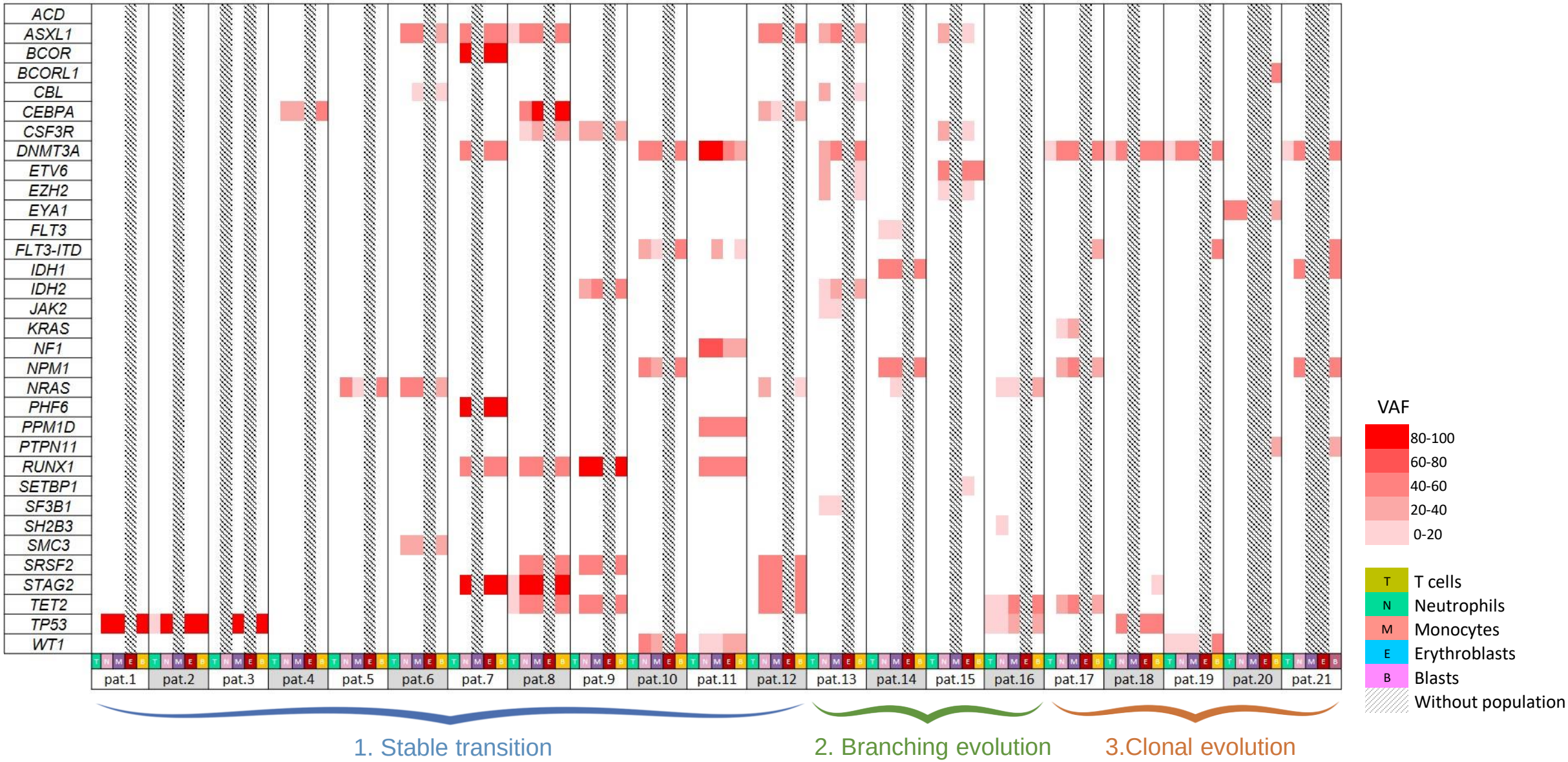

**NGS (N = 21)**

**Filter out:** synonymous; intronic; invalid-transcript, panel error; SNP's;
**Filter in:** VAF>=5% mature cells, VAF <=20% Tcells, 85%reads >=200x
48genes related to AML/MDS

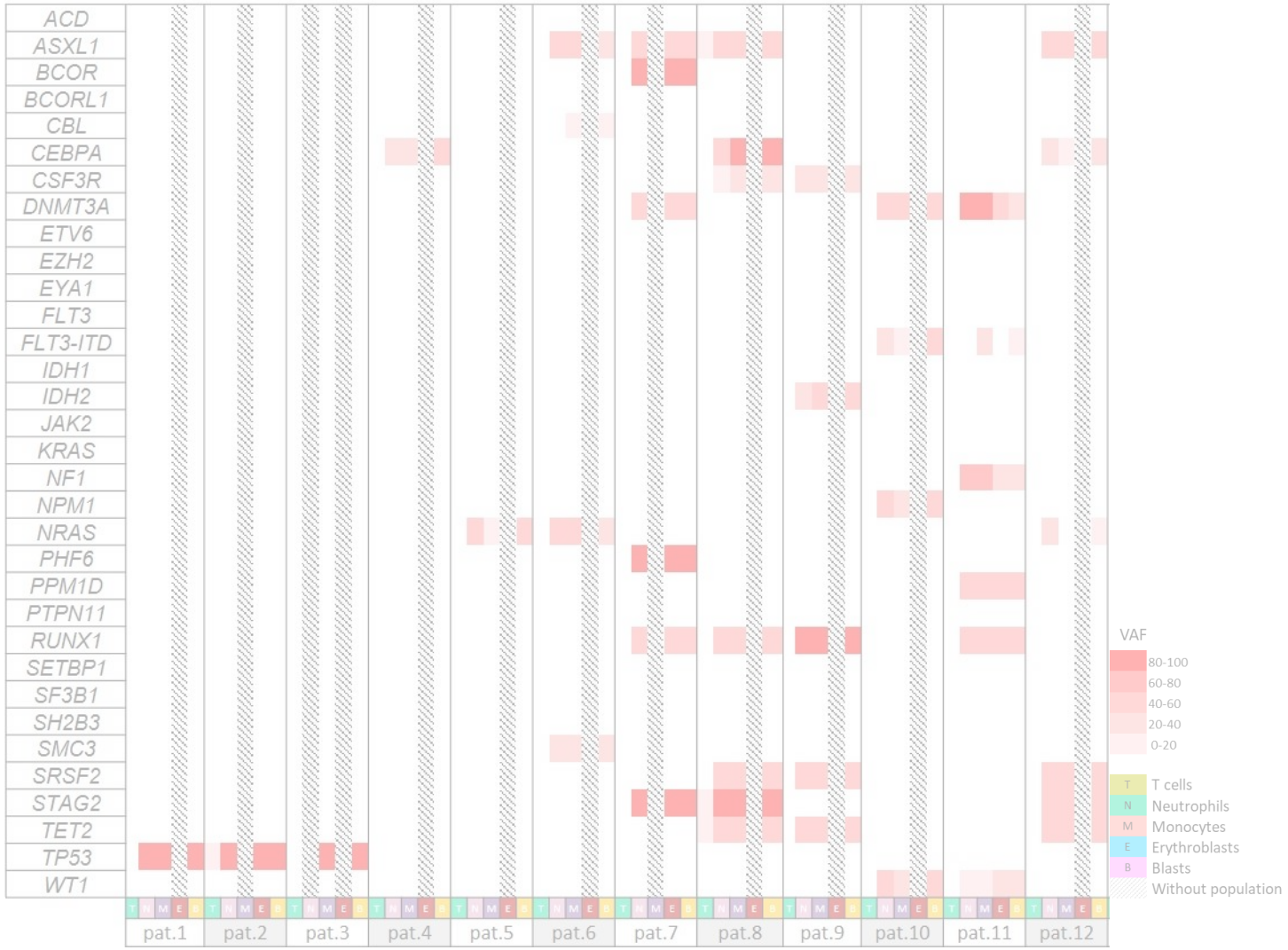
# NGS in dysplastic cells and blasts at diagnosis, isolated by FACS



# Three evolutionary patterns of leukemogenesis

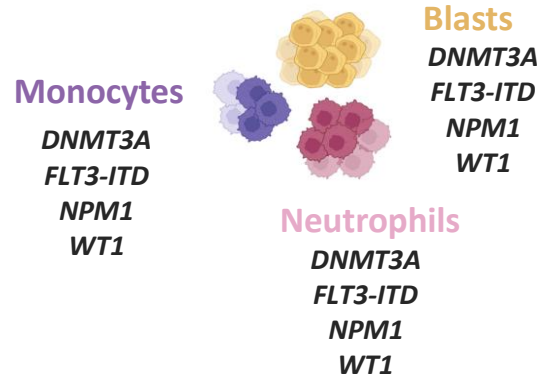


# 1. Stable transition (n = 12/21)

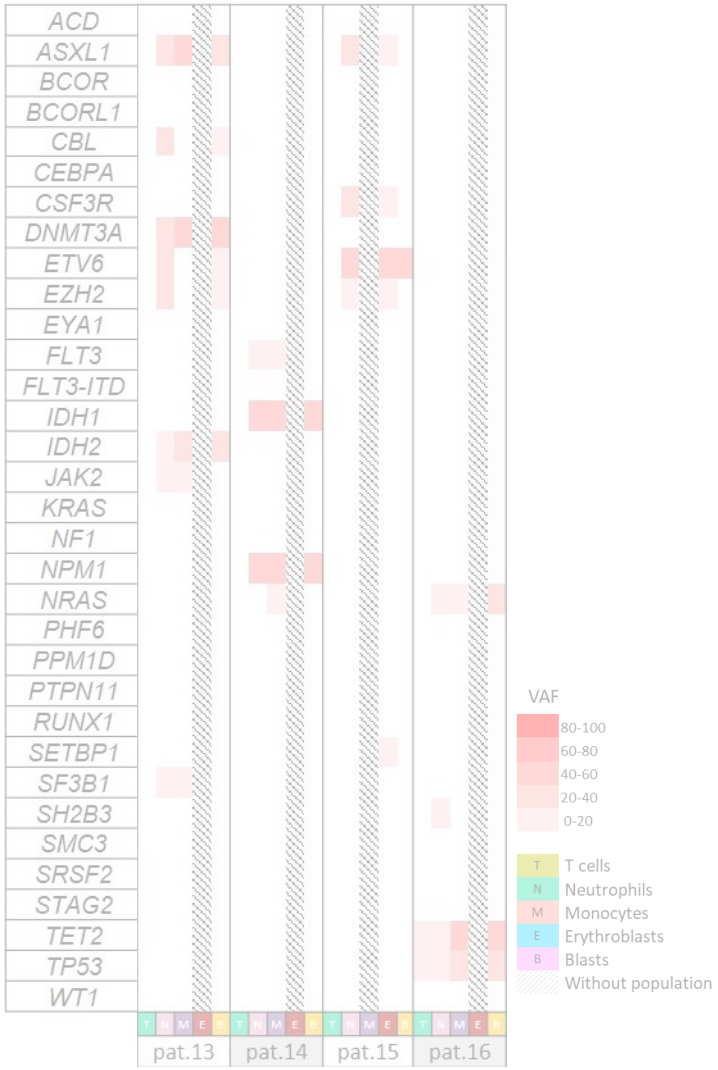



Identical mutational landscapes in blasts and residual mature dysplastic cells
(*ASXL1*, *RUNX1*, *TP53)*

**Patient 10**



# 2. Branching evolution (n = 4/21)




Blasts originated from leukemic stem cells other than the ones driving dysplasia, due to mutations absent in blasts and present in dysplastic cells (*JAK2*, *KRAS*, *NRAS*)

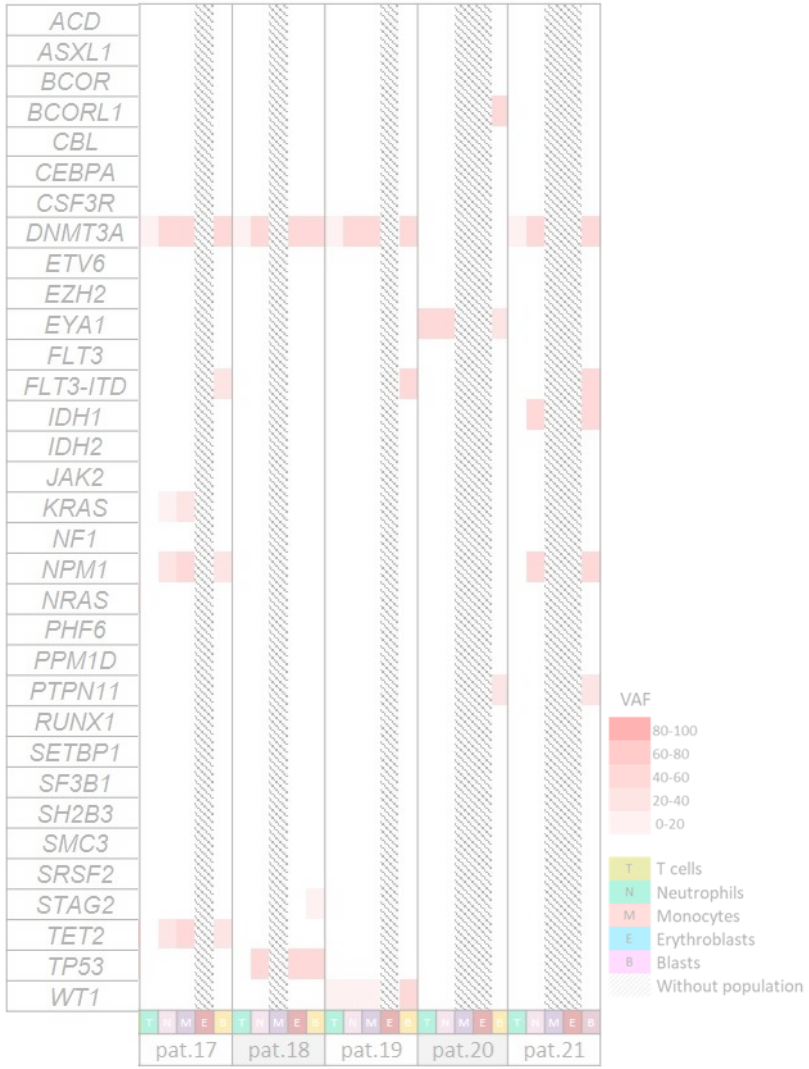
**Patient 14**

**Blasts**
***NPM1***
***IDH1***

**Monocytes**
***NPM1***
***IDH1***
***FLT3***
***NRAS***

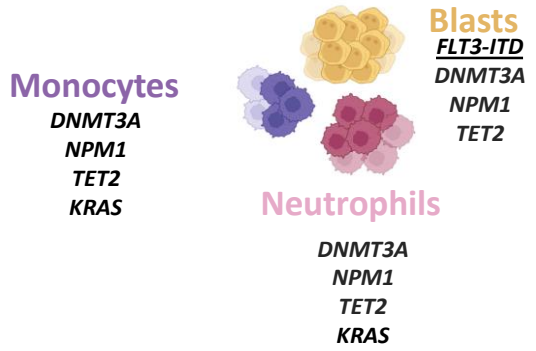
**Neutrophils**
***NPM1***
***IDH1***
***FLT3***

# 3. Clonal evolution (n = 5/21)

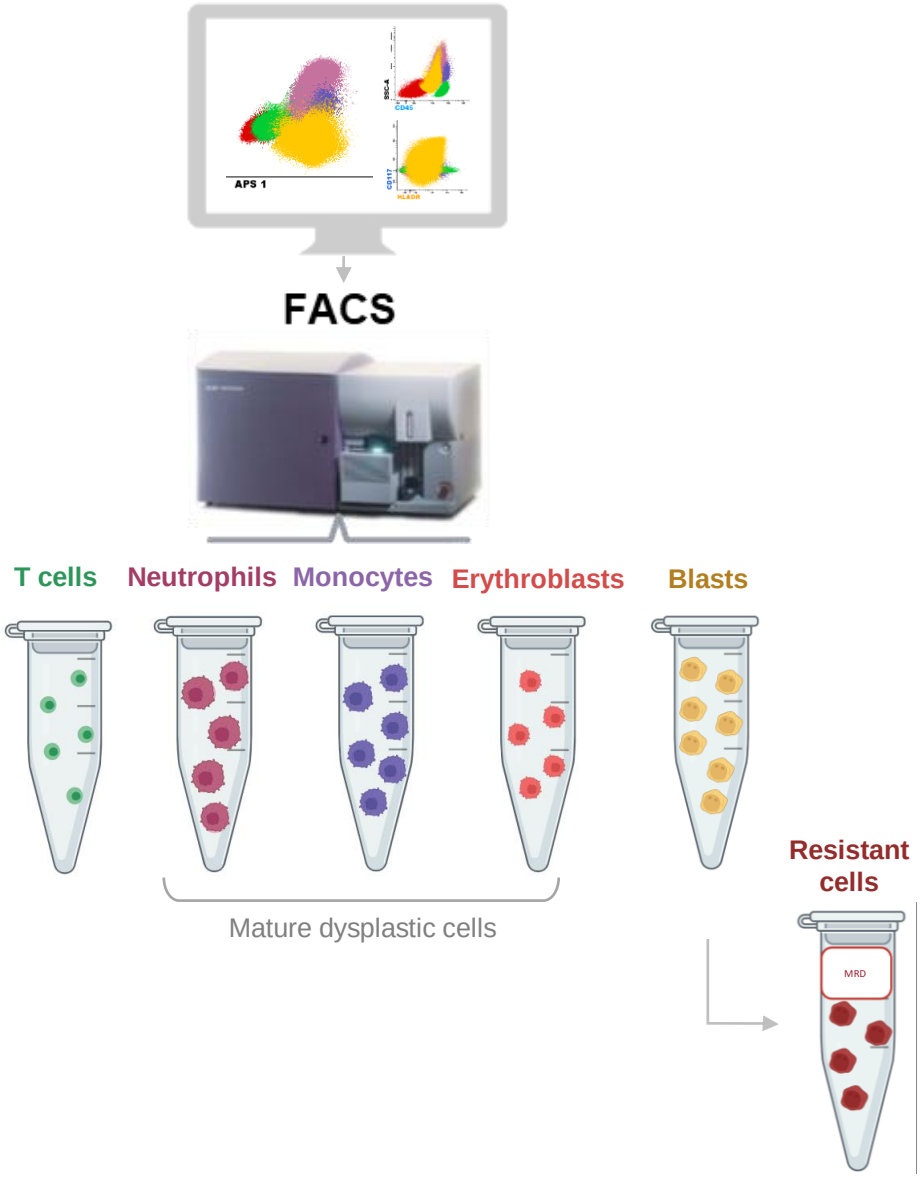



New mutations in blasts onto mutations shared between these and dysplastic cells (*FLT3-ITD*, *STAG2*)

**Patient 17**



# Genetic characterization of dysplastic cells, blasts at diagnosis and MRD cells




**WES (N = 6)**

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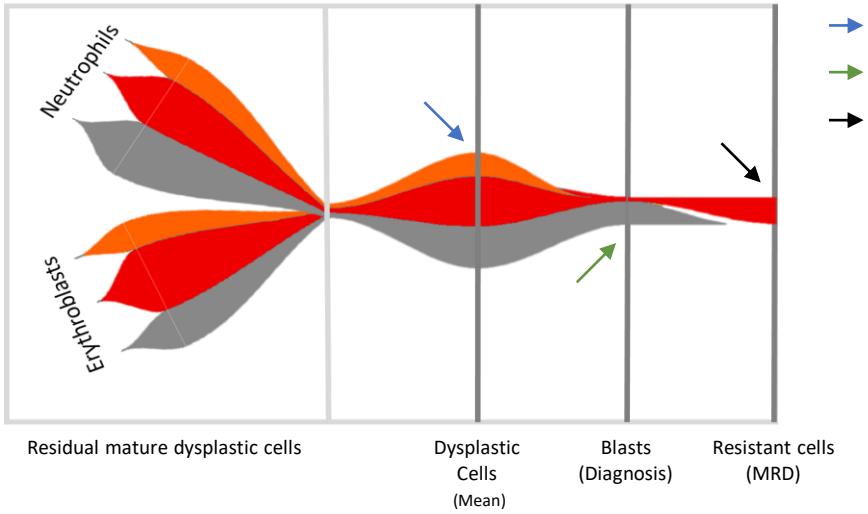
# Three evolutionary patterns in nearly all cases (n=5/6)




*HRNR*
*ZNF587*
*MUC2*

Mutations undetectable in one or more cell types

**110 mutations detected in all cell types**

→ Mutations only in **dysplastic cells**

undetected in blasts (diagnosis) neither resistant cells (MRD)

→ Mutations shared by **dysplastic cells** and **blasts** at diagnosis

undetected in resistant cells (MRD)

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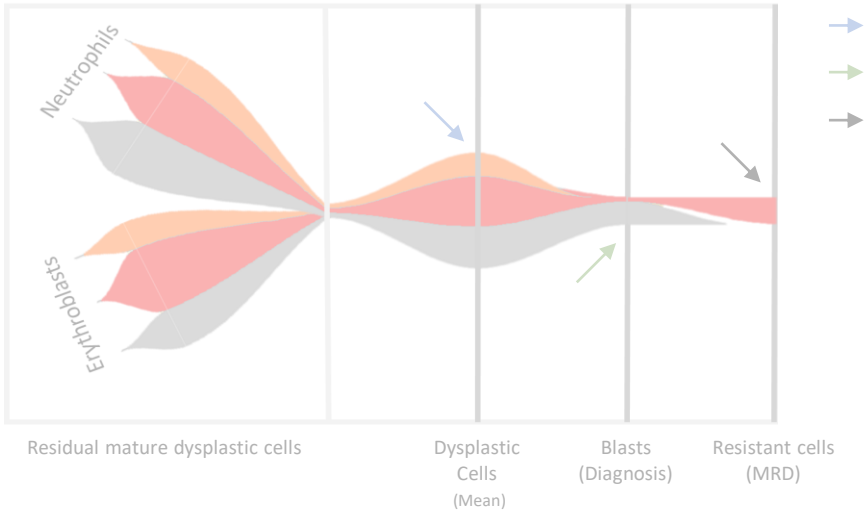


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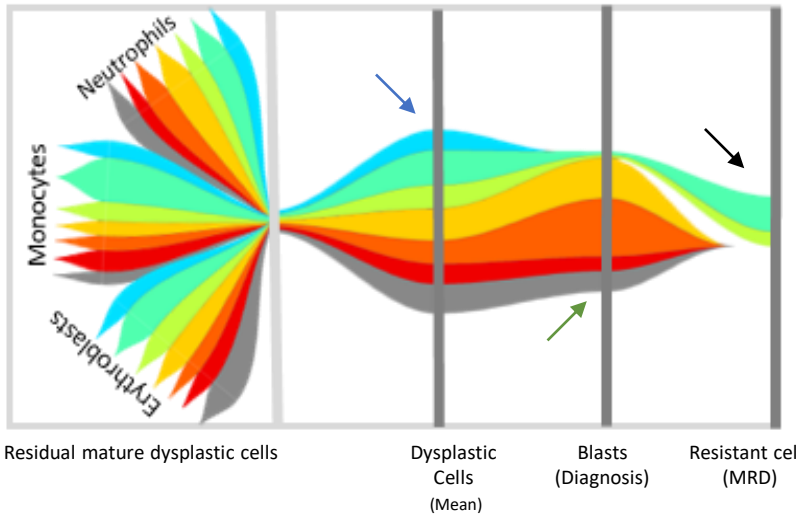
→ Mutations shared by **dysplastic cells** and **resistant cells** (MRD)




*AHNAK2*
*NBPF1*
*GGT1*
*POTEI*
*GGTLC2*
*KIR2DL3*
*KRTAP9-4*

Mutations undetectable in one or more cell types

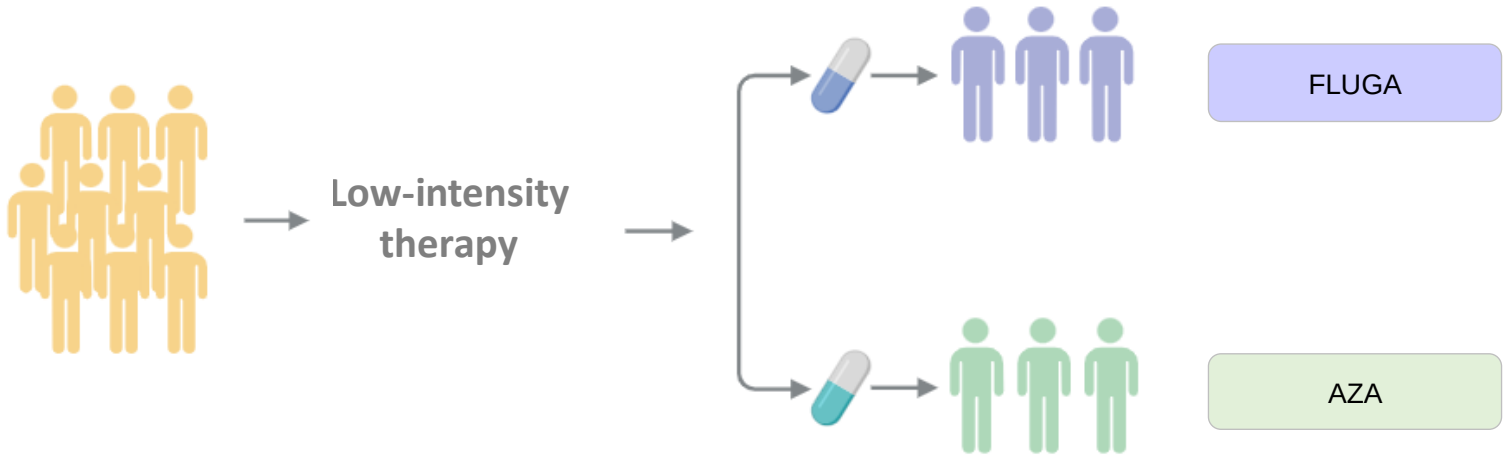
**101 mutations detected in all cell types**

# MRD in elderly AML patients

- AML incidence is higher in elderly

  Even those patients who tolerate intensive induction chemotherapy and achieve CR have a poor outcome

- Detection of MRD refines outcome prediction of younger AML patients
- MRD in elderly AML has been poorly investigated due to the reluctance of treating older patients with intensive chemotherapy, together with the renewed interest in low-intensity therapy



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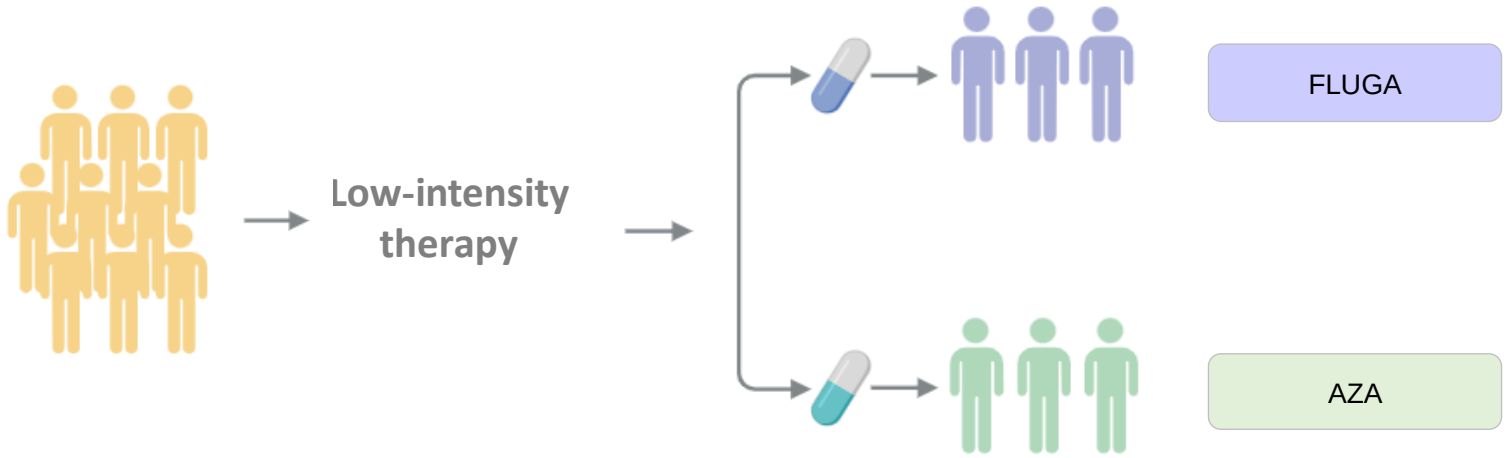
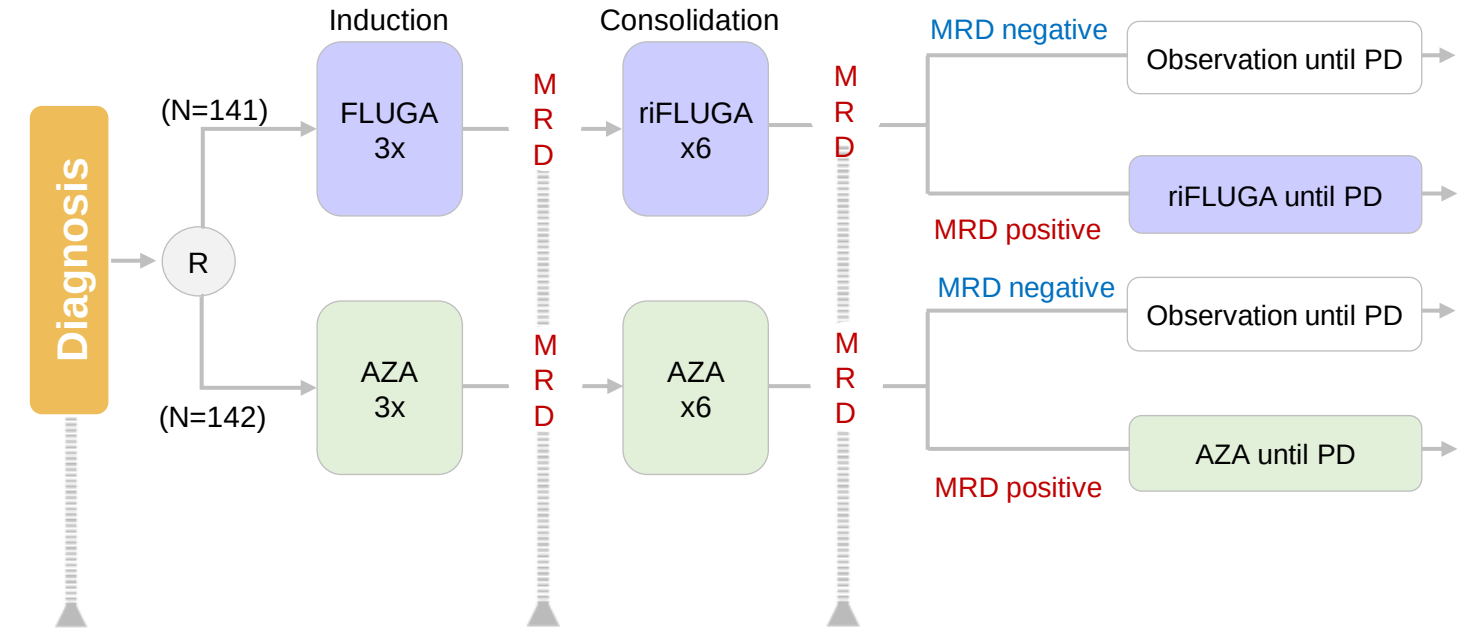



Investigate the role of MRD in refining CR and treatment duration in elderly AML patients randomized to semi-intensive chemotherapy vs HMA

# PETHEMA-FLUGAZA phase III clinical trial[1]




1. Vives S, et al. Cancer. 2021;127(12):2003-2014.

# MRD status was the only factor with independent prognostic value

| Risk factor | CIR | | RFS | | OS | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | P value | HR (95% CI) | P value | HR (95% CI) | P value |
| **Genetics** | 1.56 (0.90 – 2.71) | .113 | 1.47 (0.81 – 2.69) | .208 | 1.09 (0.60 – 2.00) | .769 |
| **Treatment** | 1.28 (0.75 – 2.18) | .361 | 1.70 (0.99 – 2.92) | .052 | 1.17 (0.68 – 2.02) | .570 |
| **MRD** | 2.95 (1.48 – 5.90) | .002 | 3.45 (1.60 – 7.45) | .002 | 1.85 (0.91 – 3.79) | .090 |

**Genetics:** adverse **vs** intermediate/favorable risk

**Treatment:** fludarabine and cytarabine **vs** azacitidine

**MRD:** positive **vs** negative

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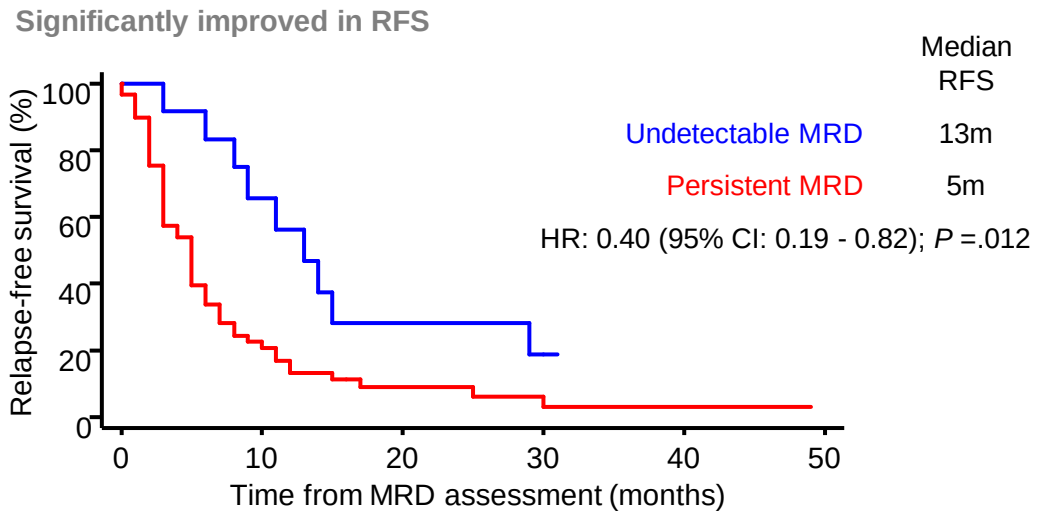
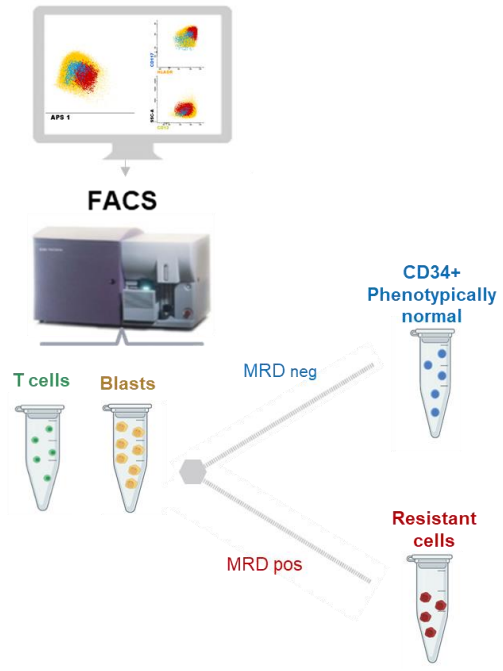
# AML patients achieving MRD- after semi-intensive therapy or HMA have lower risk of relapse



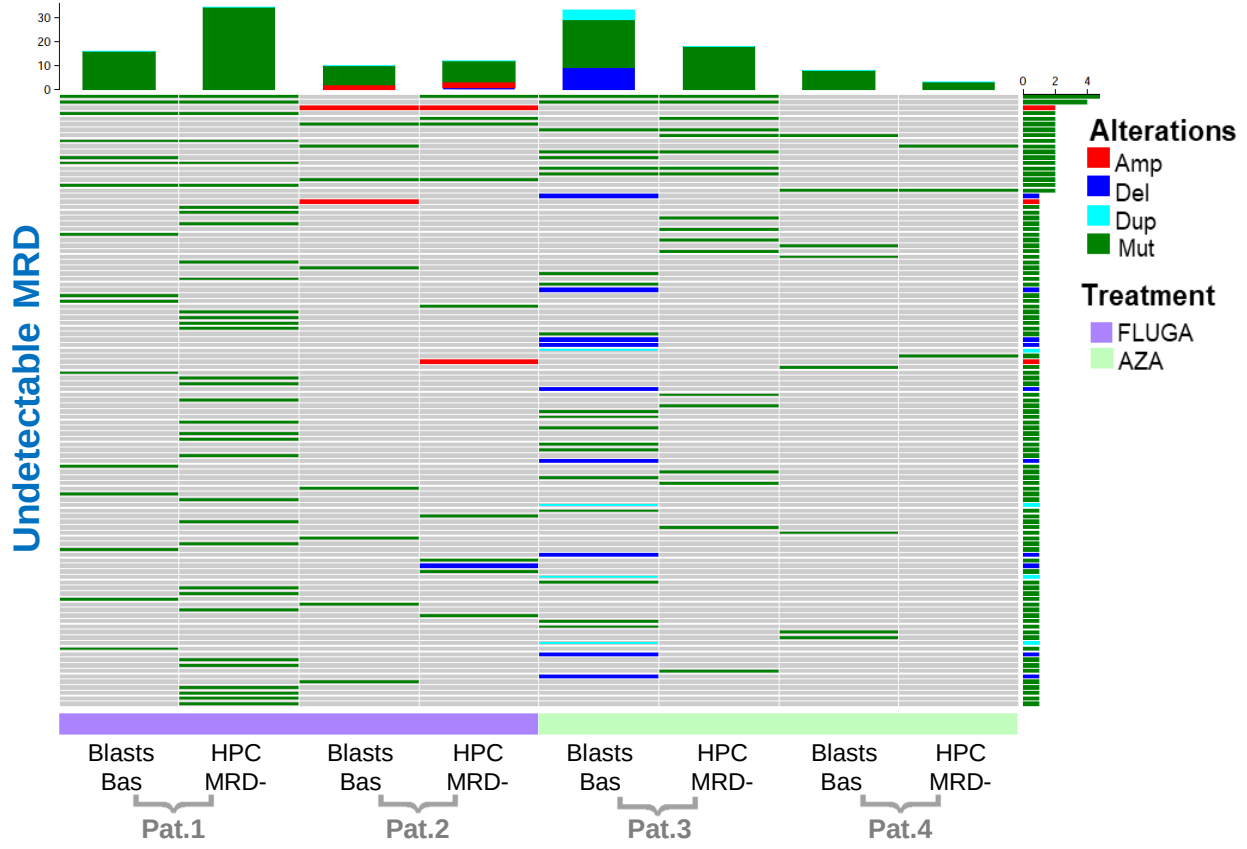

**Only 2/13 patients with MRDneg remaining relapse-free and alive**

# Do phenotypically normal CD34+ progenitors, in MRDneg patients, contain cells with leukemic-initiating-potential?

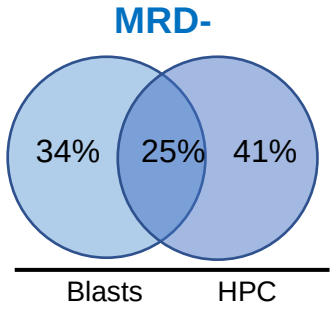


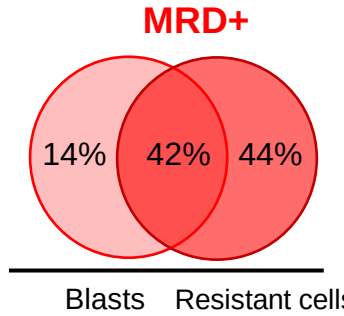

**WES (N = 10)**

Investigate the presence of genetic MRD




Mutations + CNA

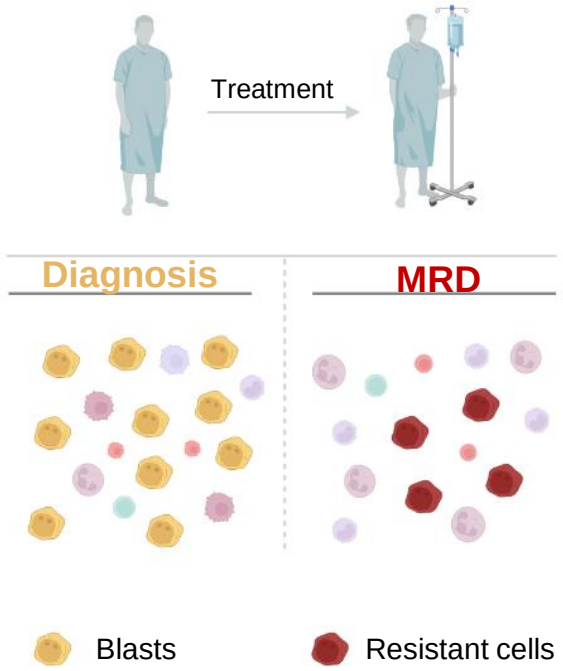


# Therapy resistance and relapse

- A better understanding of why therapies are unable to eradicate these residual leukemic cells could be relevant, particularly in elderly AML patients

- Scarce biological data about the mechanisms of MRD resistance because it requires
  patient-matched longitudinal samples
  ability to detect and isolate resistant cells after pre-specified time points and in a setting of homogenous treatment



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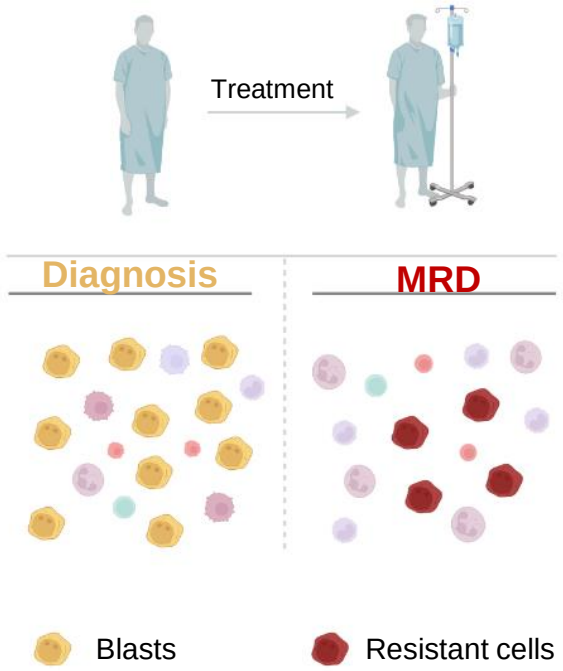
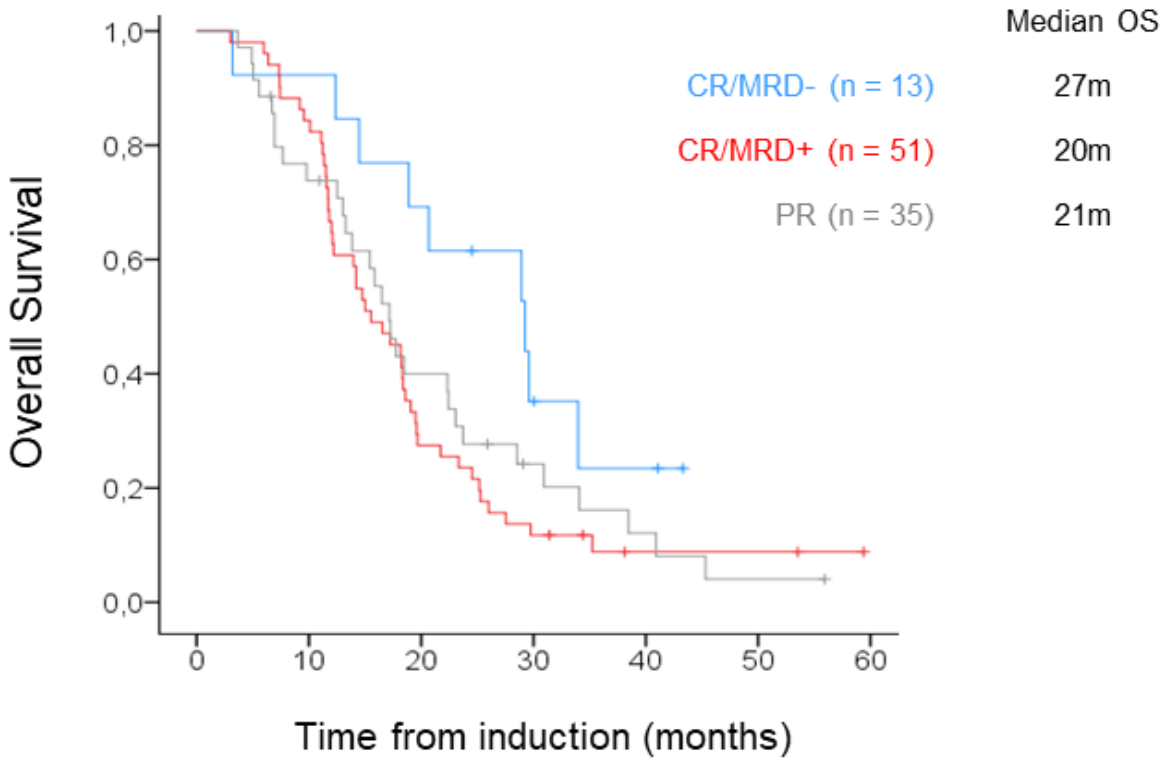



Uncover mechanisms of MRD resistance by comparing the transcriptional and genomic profile of patient-matched leukemic cells at diagnosis and after treatment

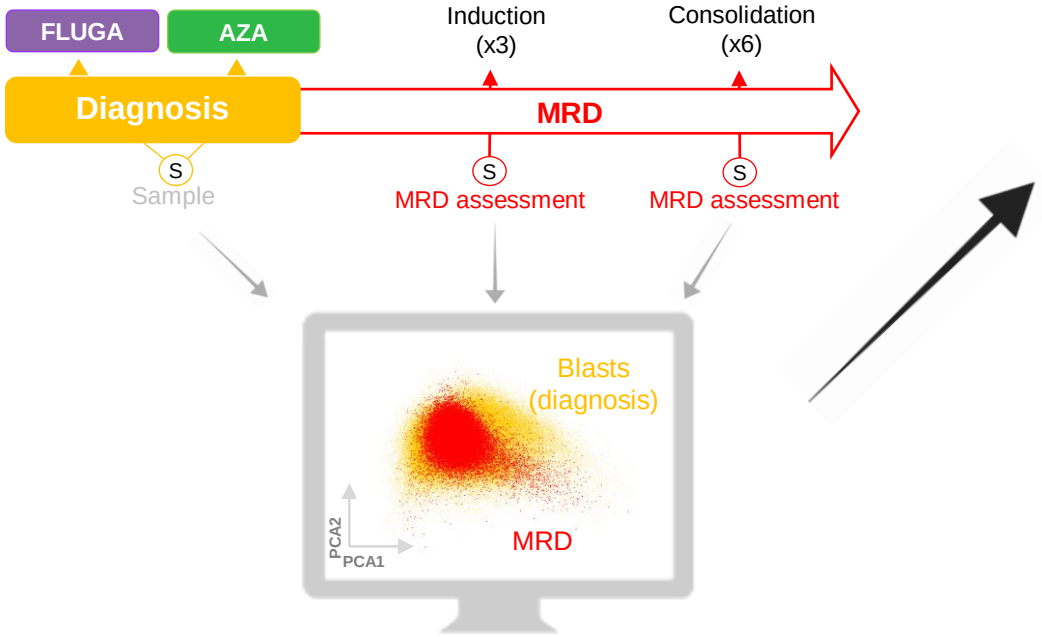
# No differences in OS between patients in PR and CR/MRD+

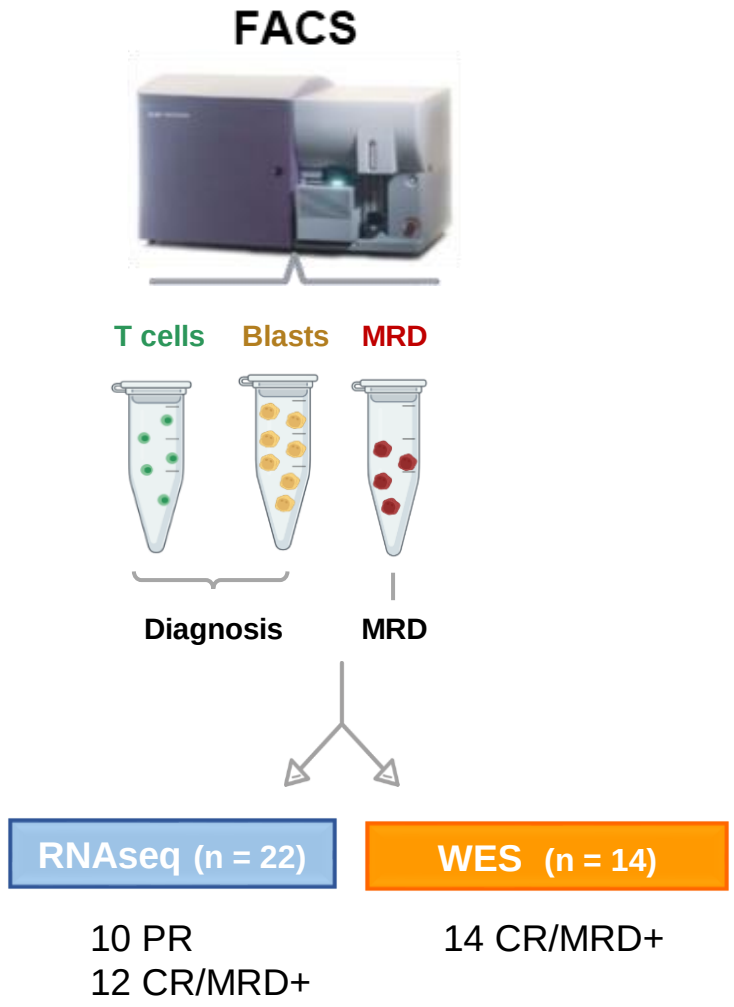
## Both showed a trend of inferior OS when compared to patients in CR/MRD-



# Transcriptomic and genetic characterization of blasts and MRD cells

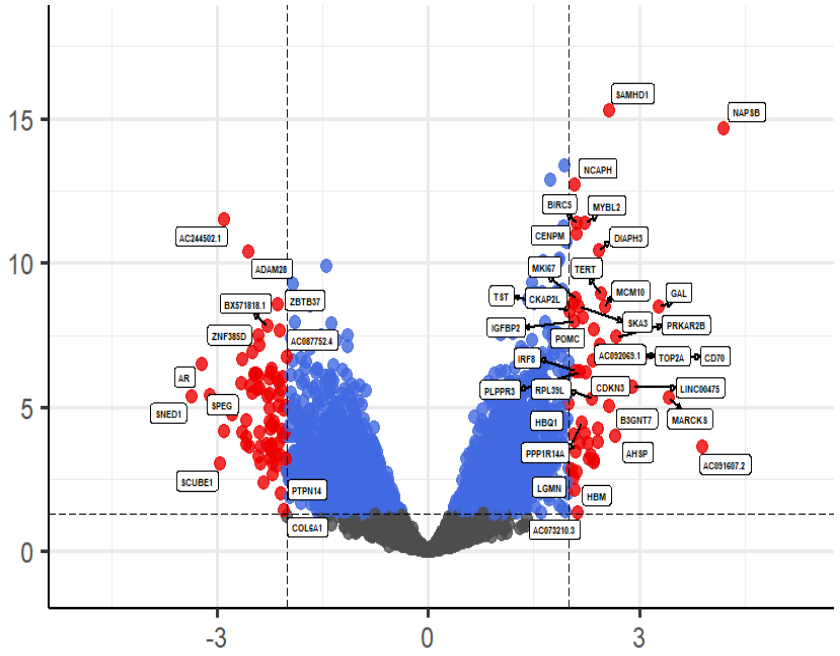
## PETHEMA-FLUGAZA phase III clinical trial[1]




1. Vives S, et al. Cancer. 2021;127(12):2003-2014.

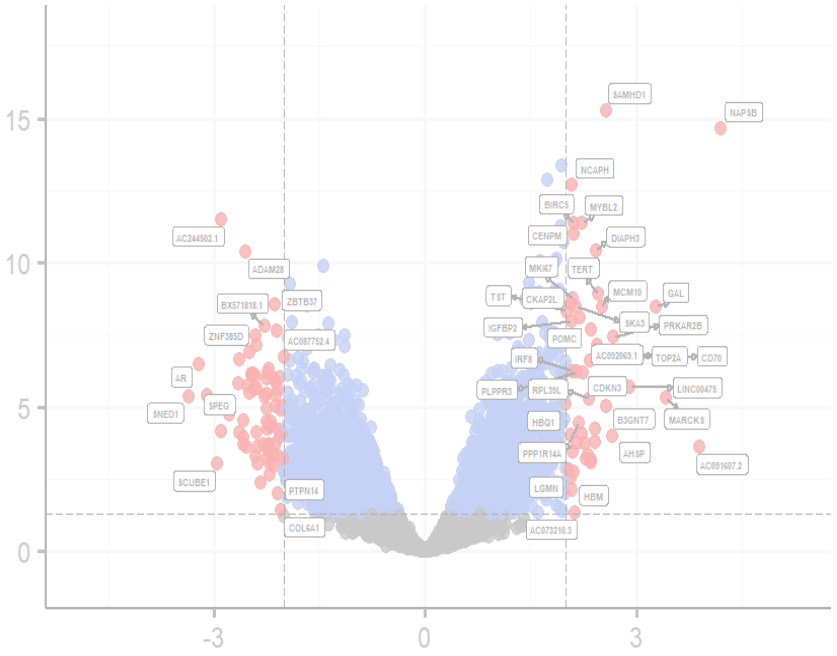
# Differentially expressed genes in treatment resistant blasts




Criteria
adjP <.05, log2FoldChange>|2|

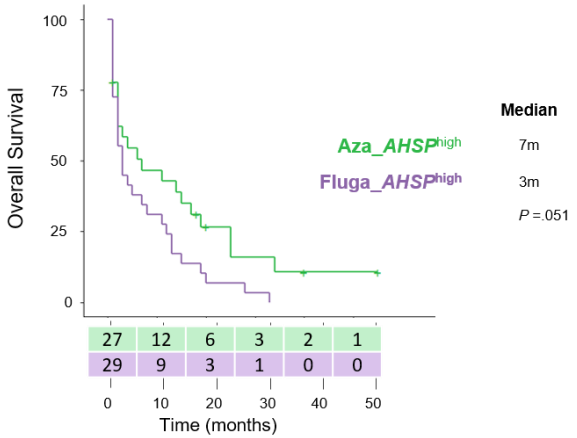
- Partial Remission *vs* Diagnosis: *PIEZO2*
- CR/MRD+ *vs* Diagnosis
  - 47 over-expressed genes
  - 70 under-expressed genes

# *ASHP* differentially expressed between treatments arms

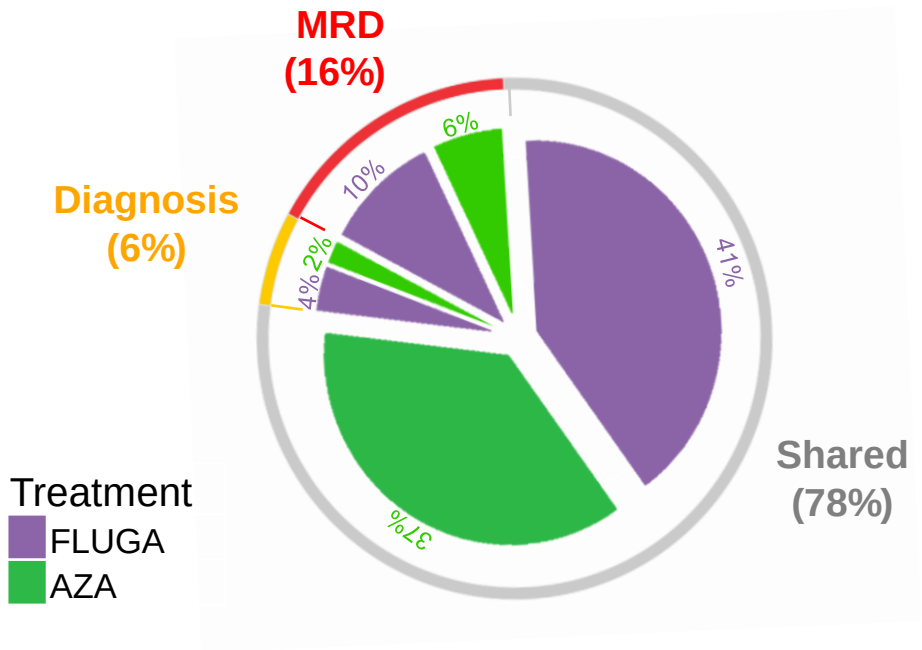


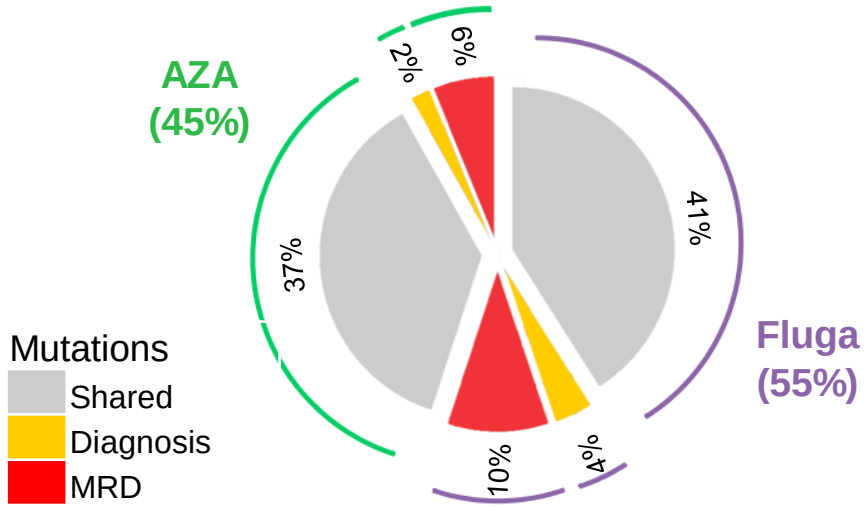

- Partial Remission *vs* Diagnosis: *PIEZO2*
- CR/MRD+ *vs* Diagnosis
  - 47 over-expressed genes
  - 70 under-expressed genes

- *AHSP* was over-expressed in patients receiving AZA but not FLUGA
- Trend for prolonged OS with AZA vs FLUGA among patients over-expressing *AHSP*



# WES of matched leukemic cells at diagnosis and after treatment

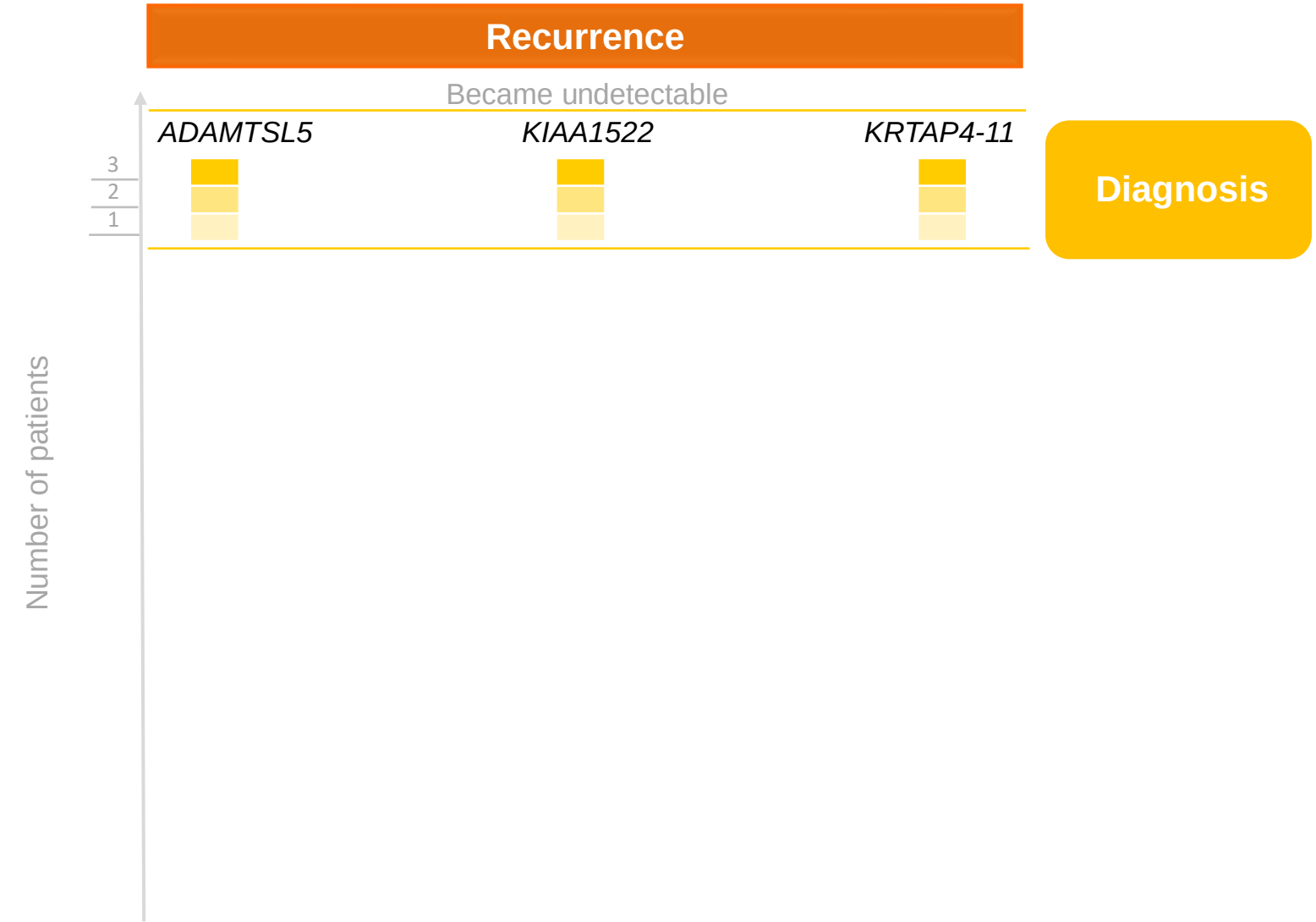



- 4,708 (78%) were detected at both time points
- 354 (6%) were present at diagnosis while absent in MRD blasts
- 992 (16%) emerged during MRD resistance

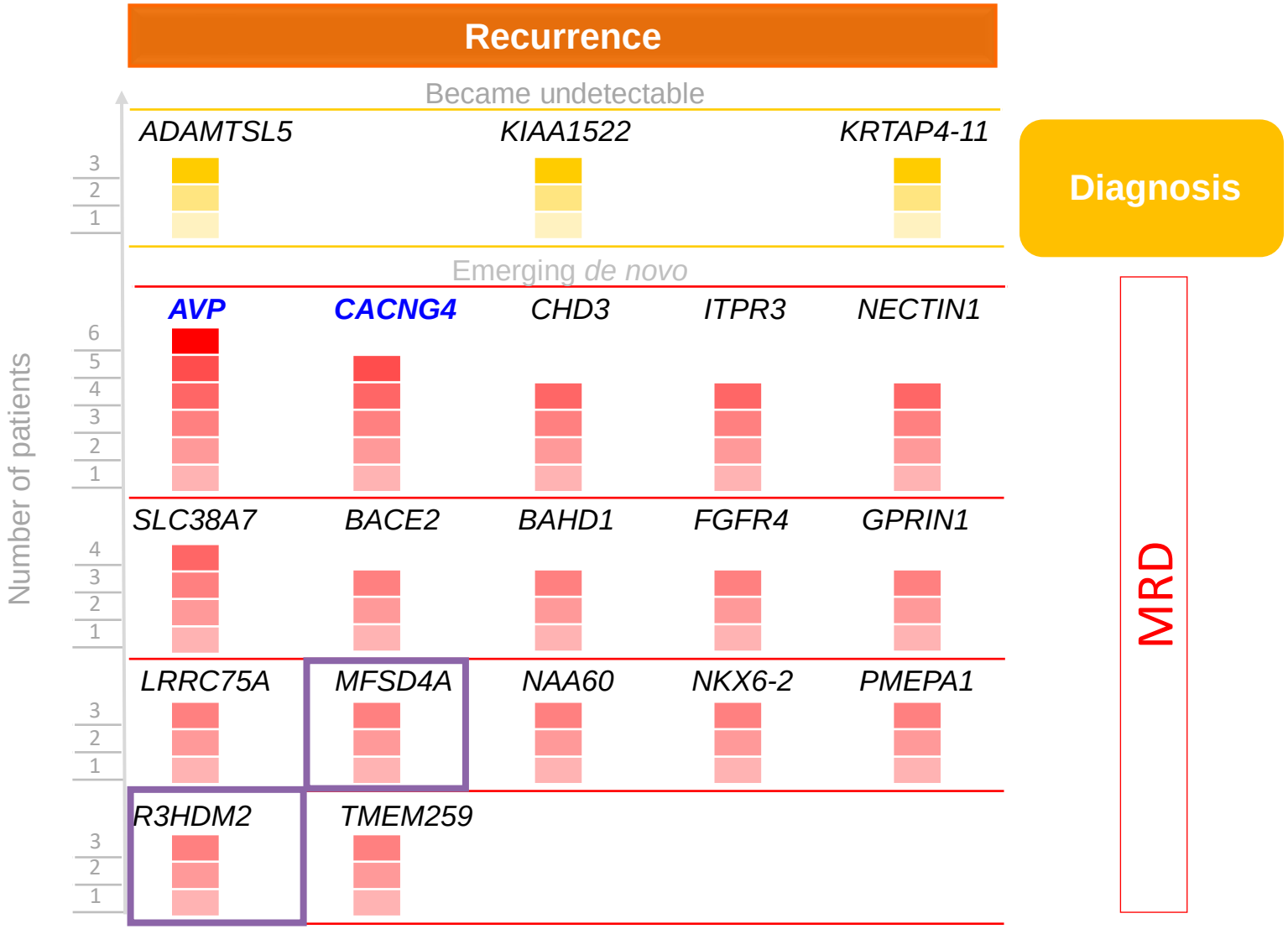
# Recurrent genes (>3 patients) associated with MRD cells

## At diagnosis that became undetectable in MRD cells (3 genes)



# Recurrent genes (>3 patients) associated with MRD cells

## Mutations emerging *de novo* in MRD cells (17 genes)



# Discussion

- Dysplastic cells were detected by MFC in 82% of newly diagnosed patients, indicating that it could be possible to reconstruct leukemogenesis at the onset of AML in most cases
- Three evolutionary patterns from dysplasia to newly diagnosed AML: stable, branching and clonal evolution
- Different clonal involvement in dysplastic myelo-erythropoiesis, leukemic transformation, and chemoresistance
- Attaining undetectable MRD after semi-intensive therapy or HMA is prognostically relevant in elderly patients with AML
- HPC in patients with undetectable MRD by MFC possess extensive genetic abnormalities, almost as much as leukemic MRD cells
- PR appear to be characterized by primary resistance, whereas CR with persistent MRD is associated with the emergence of molecular traits of acquired resistance

## Manuscripts

**“Integrated flow cytometry and sequencing to reconstruct evolutionary patterns from dysplasia to acute myeloid leukemia”**
Simoes C., Chillon MC., … & Montesinos P.*, Paiva B.*; Blood Adv. 2023;7(1):167-173

**“Measurable residual disease in elderly acute myeloid leukemia: results from the PETHEMA-FLUGAZA phase 3 clinical trial”**
Simoes C.*, Paiva B.*, … & Montesinos P.; Blood Adv. 2021;5(3):760-770

**“Transcriptional and genomic characterization of measurable residual disease in acute myeloid leukemia”**
Simoes C., Villar S., …&Paiva, B.*, Montesinos P.*, British Journal of Haematology, 2023;201(6):1239-1244

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