

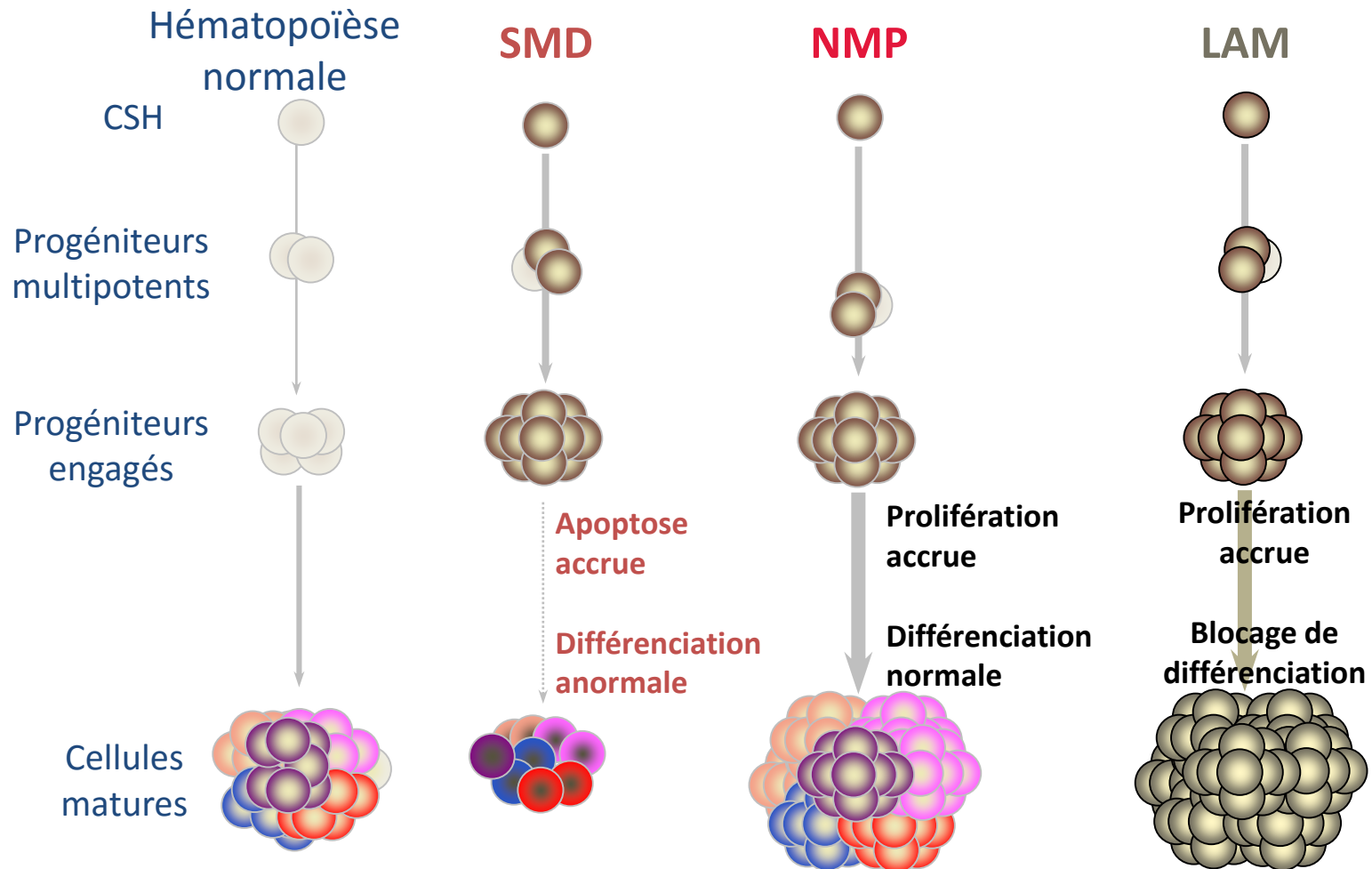
# Hiérarchisation des événements génétiques dans les hémopathies : leucémies aiguës myéloïdes

François Delhommeau

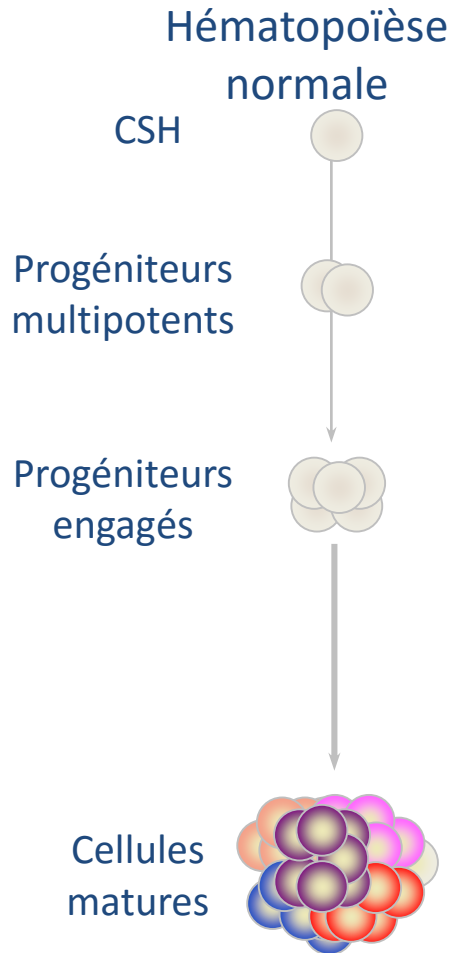
Hématologie cellulaire

Centre de recherche et Hôpital Saint-Antoine

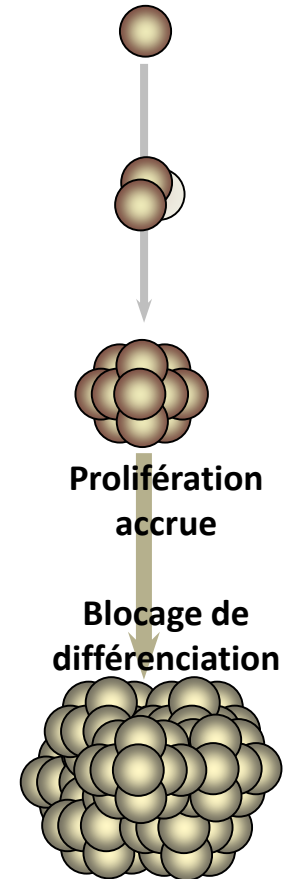
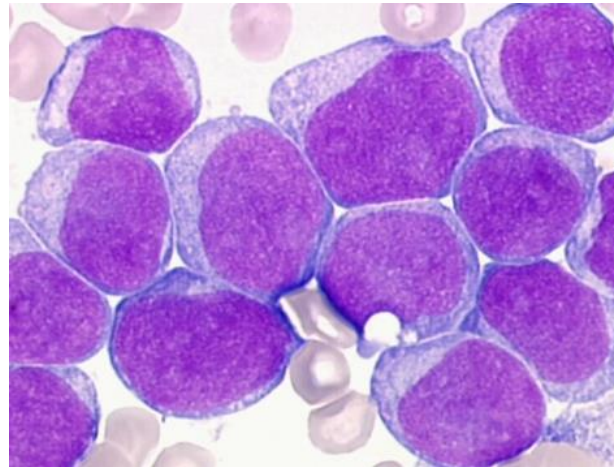
# Hémopathies myéloïdes



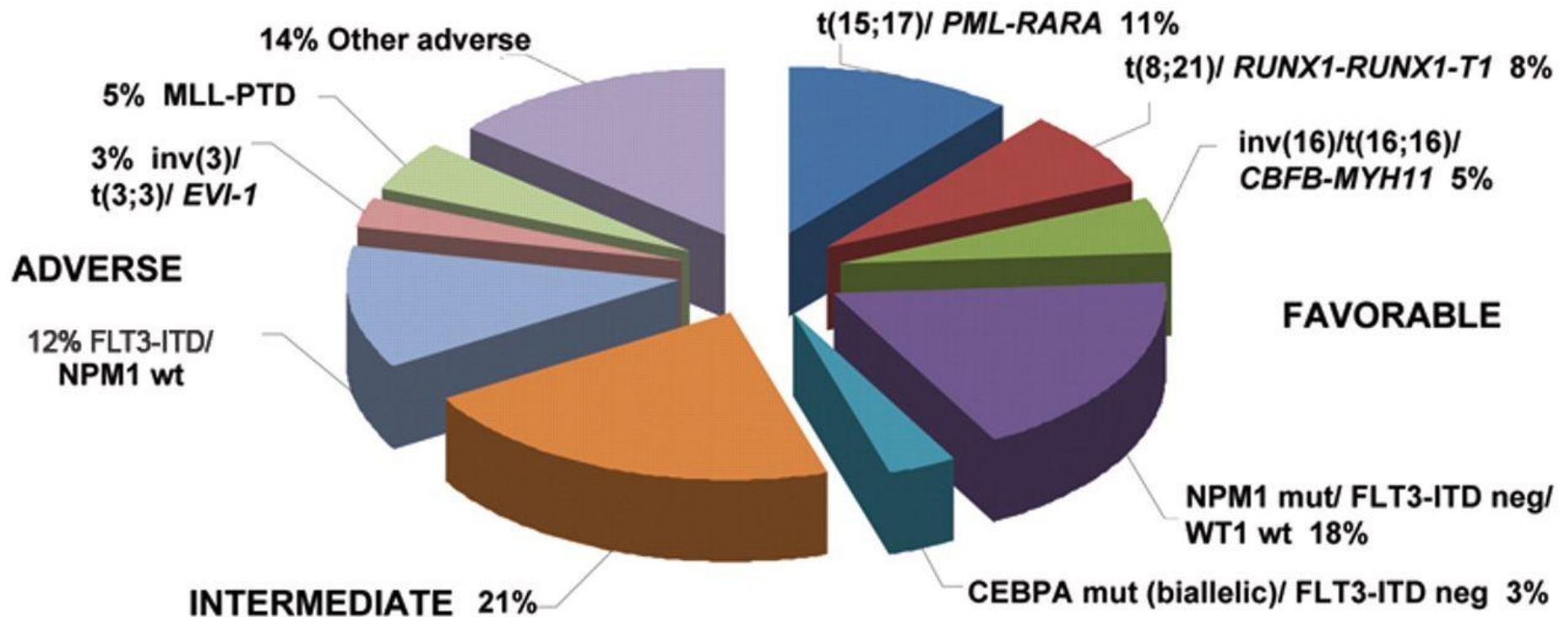
# Hémopathies myéloïdes



## LAM : Leucémies aiguës myéloïdes



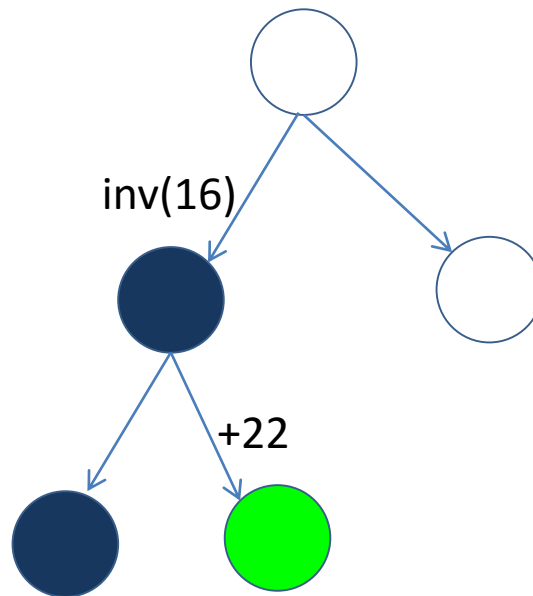
# Hétérogénéité génétique des LAM



46,XX,inv(16)(p13;q22)[9]/47,idem,+22[5]/ 46,XX[18]

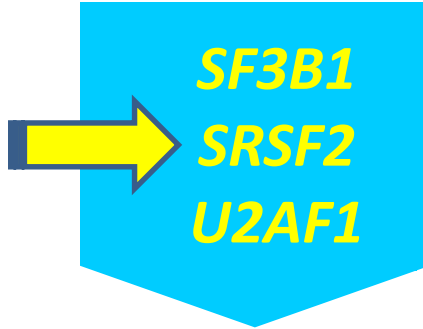
# L'évolution clonale des leucémies : un vieux truc de cytogénéticien !

46,XX,inv(16)(p13;q22)[9]/47,idem,+22[5]/ 46,XX[18]



# NGS des LAM

Machinerie d'épissage



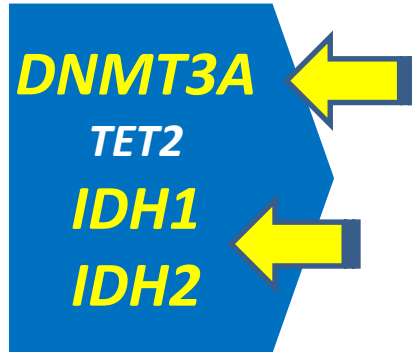
Nucléophosmine 1



Facteurs de transcription



Méthylation de l'ADN



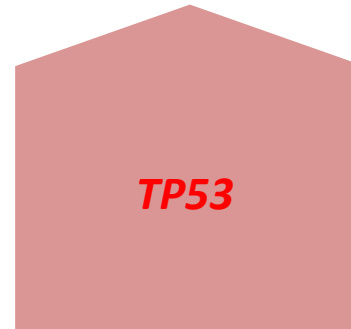
Transduction du signal



Modif. chromatine



Complexe cohésine



Suppresseurs de tumeur

Machinerie  
d'épissage



Nucléophosmine  
1



Facteurs de  
transcription



Différenciation

Prolifération

Stabilité du  
génom

Epigénétique

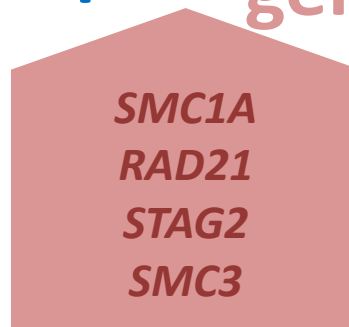
Méthylation de  
l'ADN



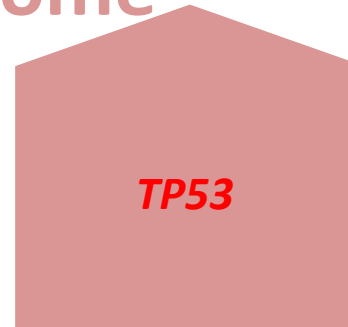
Transduction du  
signal



Modif.  
chromatine



Complexe  
cohésine



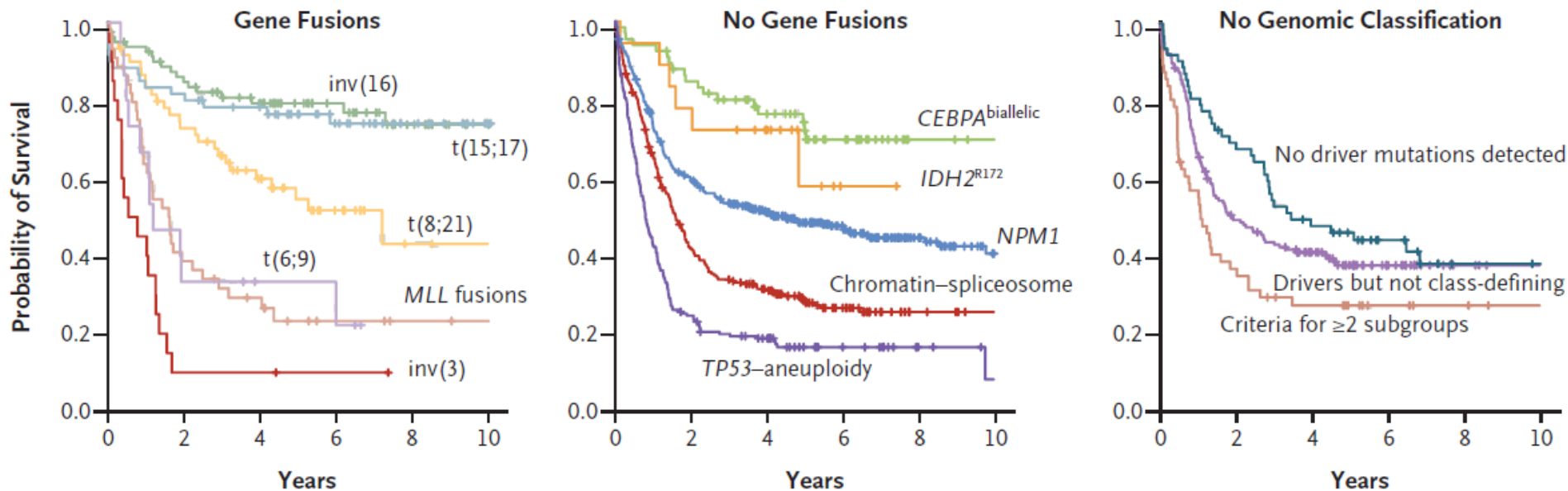
Suppresseurs de  
tumeur



# Genomic Classification and Prognosis in Acute Myeloid Leukemia

Elli Papaemmanuil, Ph.D., Moritz Gerstung, Ph.D., Lars Bullinger, M.D., Verena I. Gaidzik, M.D., Peter Paschka, M.D., Nicola D. Roberts, B.Sc., Nicola E. Potter, Ph.D., Michael Heuser, M.D., Felicitas Thol, M.D., Niccolo Bolli, M.D., Ph.D., Gunes Gundem, Ph.D., Peter Van Loo, Ph.D., Inigo Martincorena, Ph.D., Peter Ganly, B.M., B.Ch., Ph.D., Laura Mudie, B.Sc., Stuart McLaren, B.Sc., Sarah O'Meara, B.Sc., Keiran Raine, M.Sc., David R. Jones, M.Sc., Jon W. Teague, B.Sc., Adam P. Butler, B.Sc., Mel F. Greaves, Ph.D., Arnold Ganser, M.D., Konstanze Döhner, M.D., Richard F. Schlenk, M.D., Hartmut Döhner, M.D., and Peter J. Campbell, M.B., Ch.B., Ph.D.

A

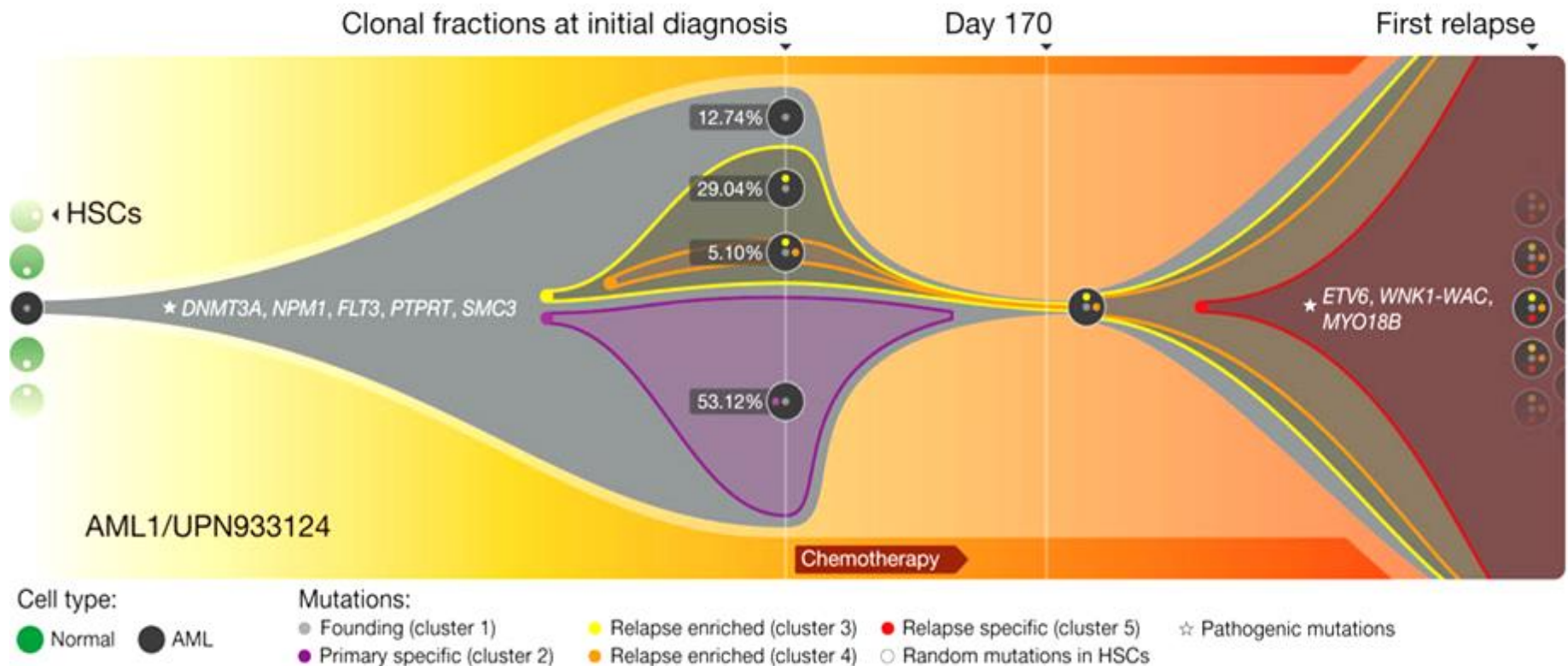


# LAM et évolution clonale

## Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing

Li Ding<sup>1,2\*</sup>, Timothy J. Ley<sup>1,3,4\*</sup>, David E. Larson<sup>1</sup>, Christopher A. Miller<sup>1</sup>, Daniel C. Koboldt<sup>1</sup>, John S. Welch<sup>3</sup>, Julie K. Ritchey<sup>3</sup>,

Reséquençage  
Populations de cellules  
Quantitatif (nombre de reads)



# Hématopoïèse clonale pré-leucémique

## CHIP (Clonal hematopoiesis of indeterminate potential)

The NEW ENGLAND JOURNAL of MEDICINE

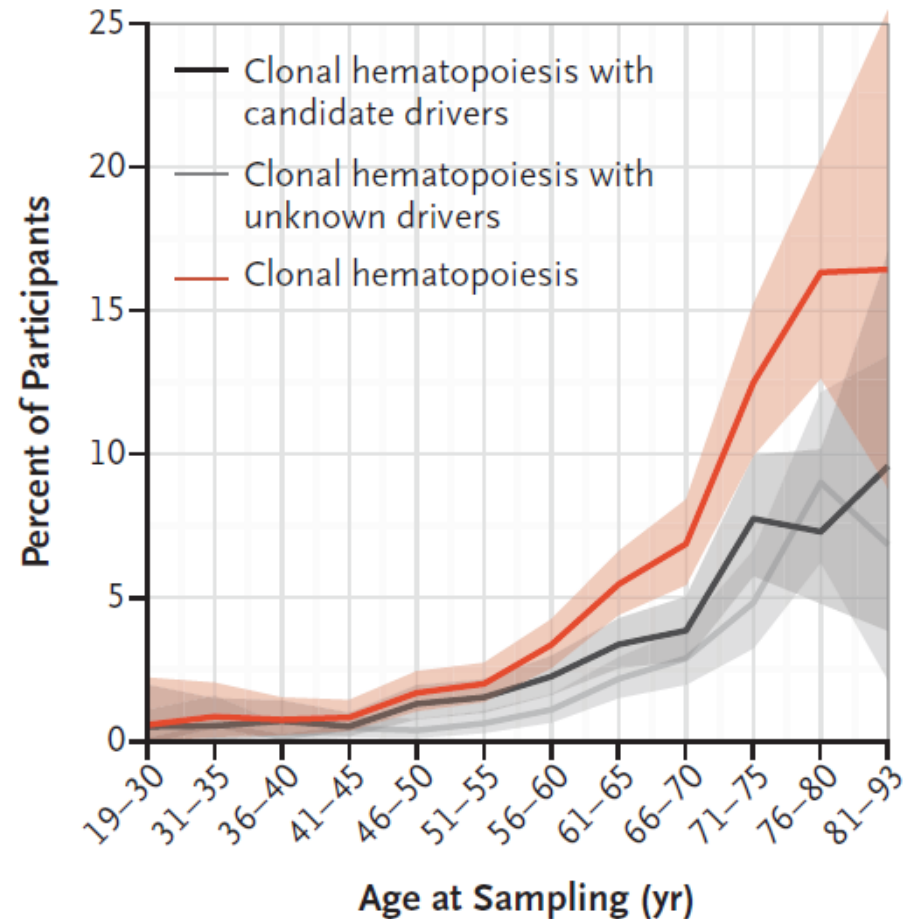
ORIGINAL ARTICLE

### Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Köhler, Ph.D., Robert E. Handsaker, B.S.,

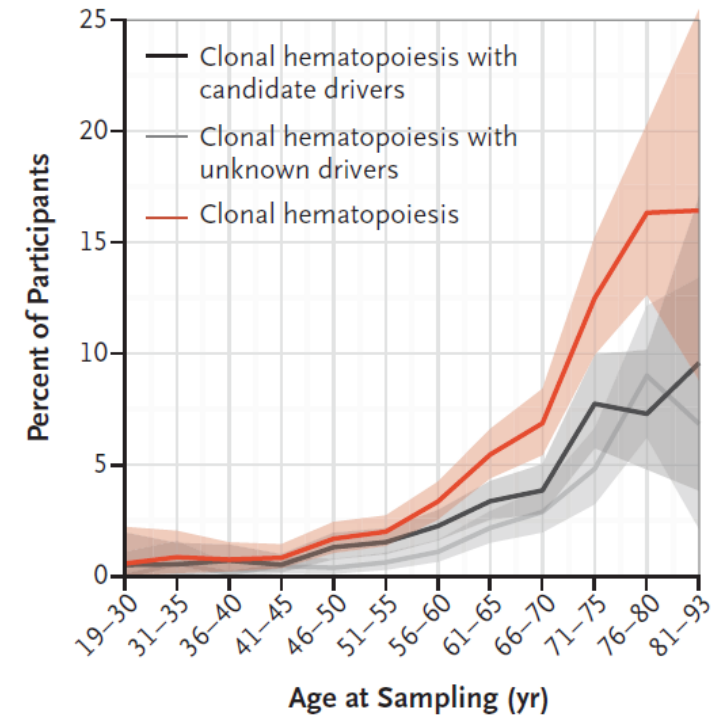
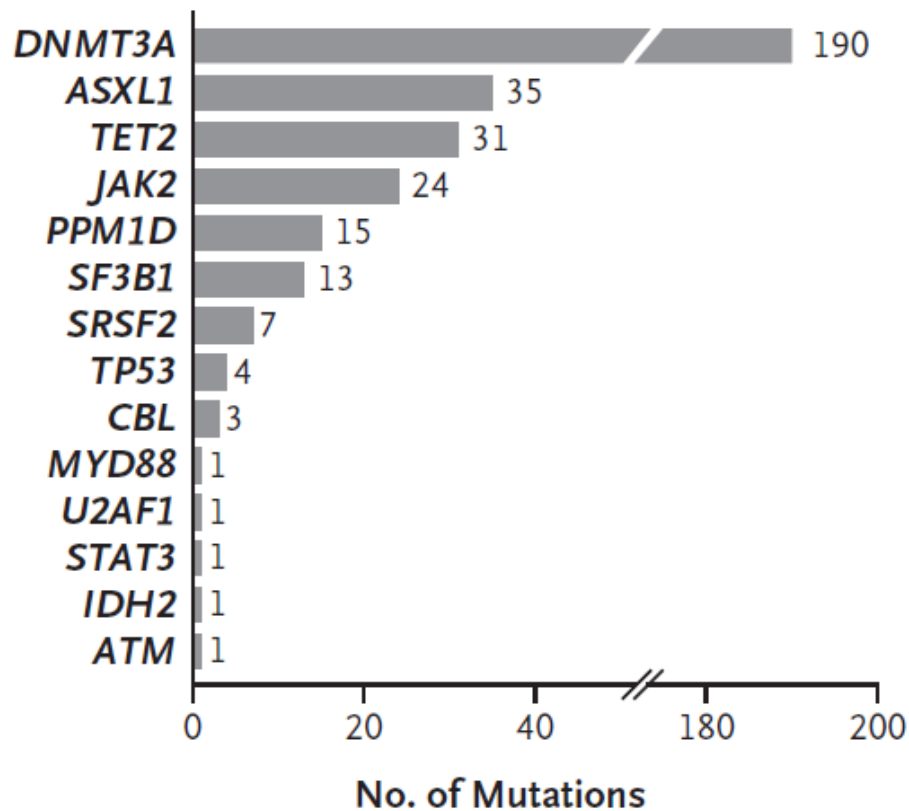
Clone sanguin chez individus sains.

(30000 exomes sur 3 études)



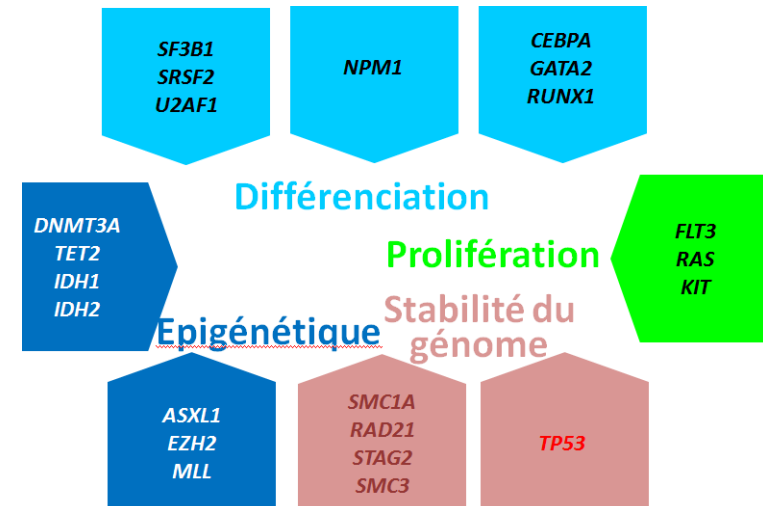
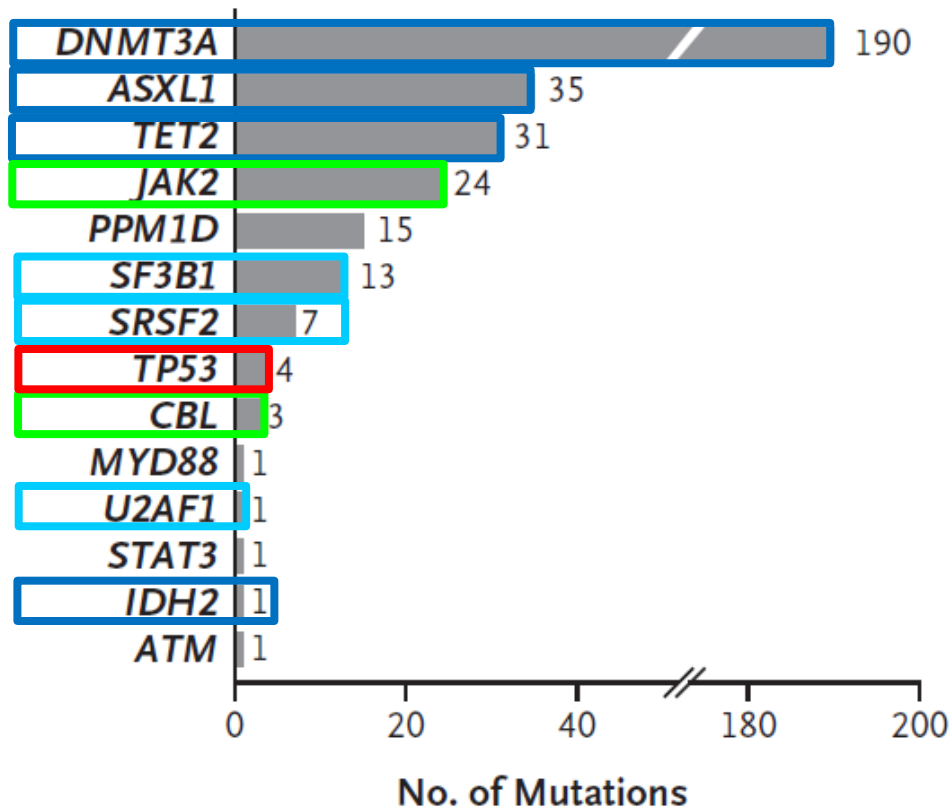
## Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Kähler, Ph.D., Robert E. Handsaker, B.S.,

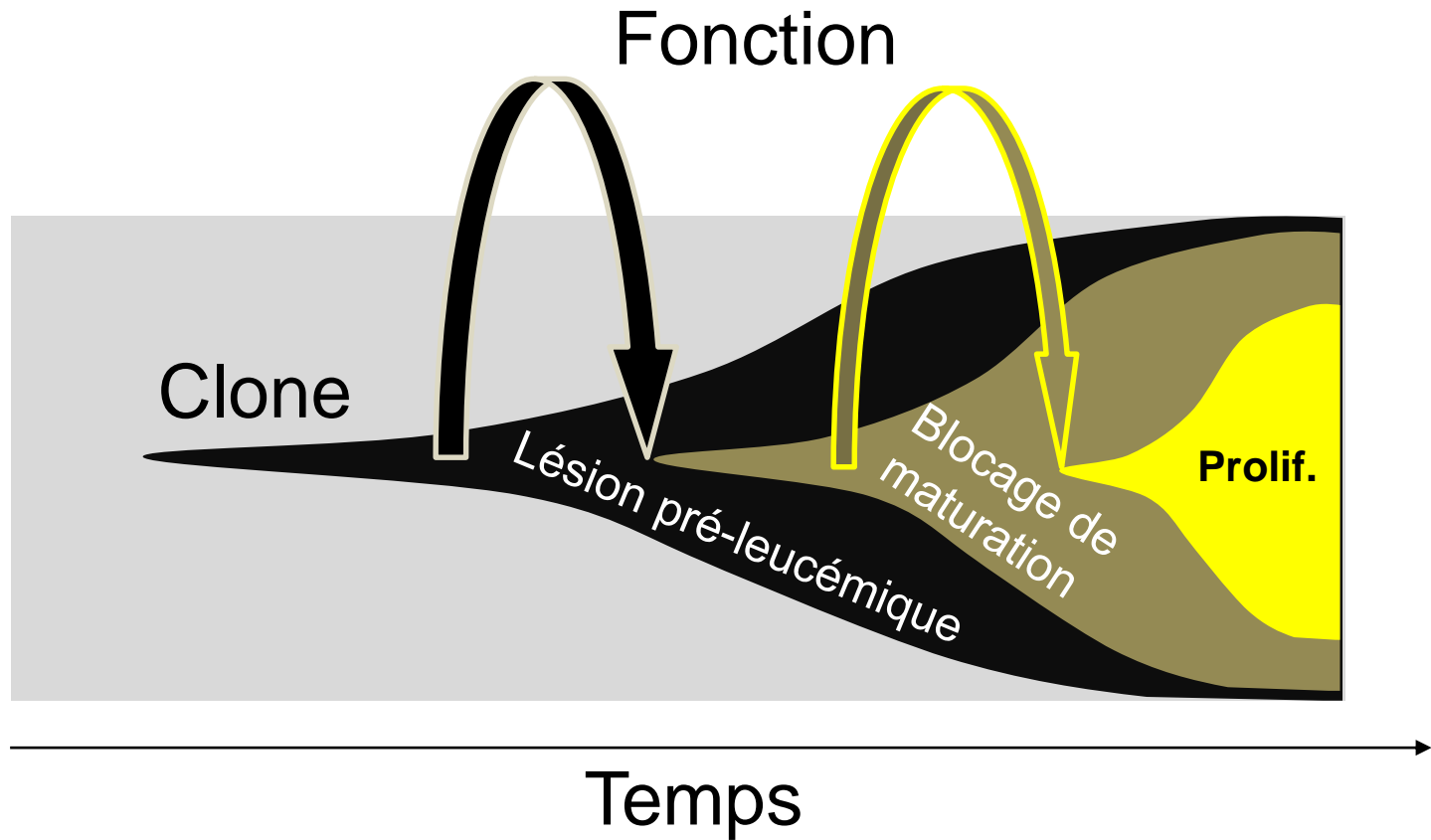


# Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Kähler, Ph.D., Robert E. Handsaker, B.S.,



# Hiérarchie fonctionnelle / temporelle ?



quelles lésions peuvent répondre à la définition de lésion pré-leucémique?

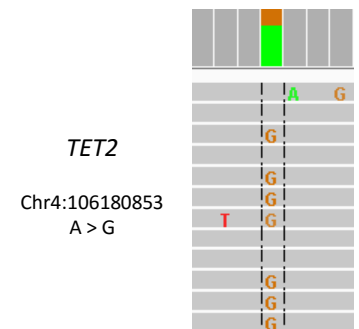
# Clone pré-leucémique : première question

- Premières lésions acquises par le clone: parfois des années (décennies?) avant le diagnostic de LAM

→ **Ordre d'acquisition des événements: phylogénie clonale**

# Approche intégrée de l'architecture clonale des LAM

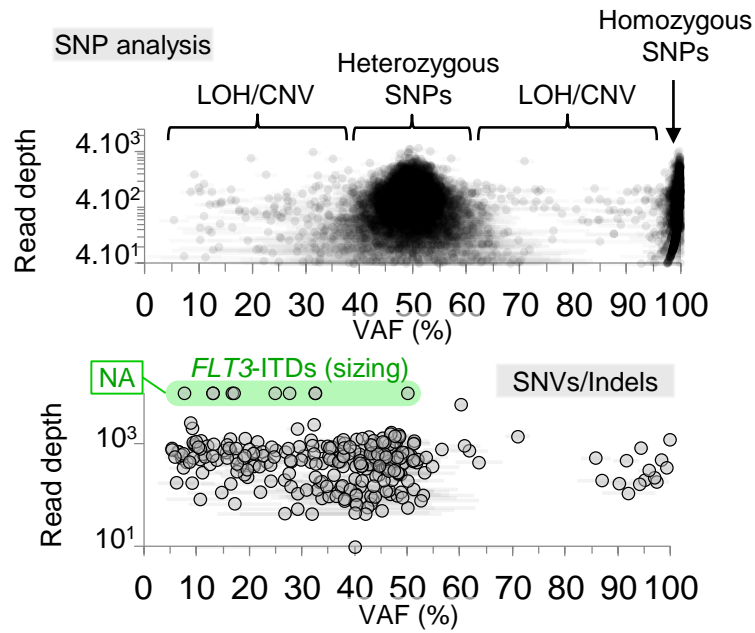
- Reséquencage ciblé
  - Panel de **122 gènes** (Haloplex)
  - Miseq (Illumina)
- Données de cytogénétique et Biologie moléculaire conventionnelle





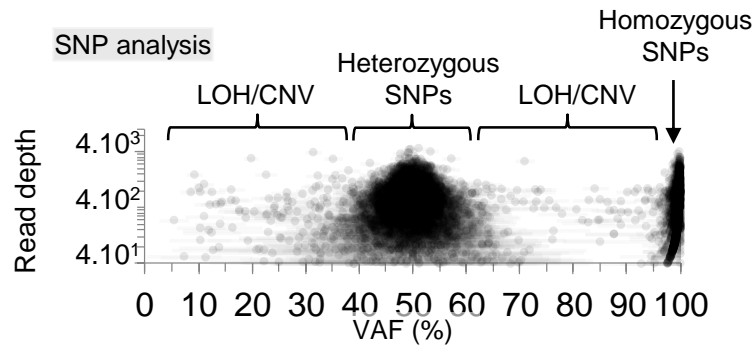
Prise en compte des Pertes d'hétérozygotie

Prise en compte des fréquences d'allèle mutés



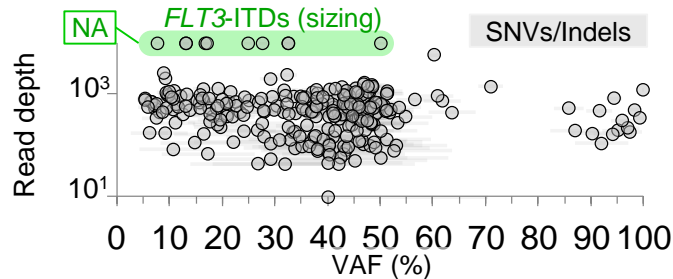
Variant Allele Frequencies (VAF)

Prise en compte des Pertes d'hétérozygotie

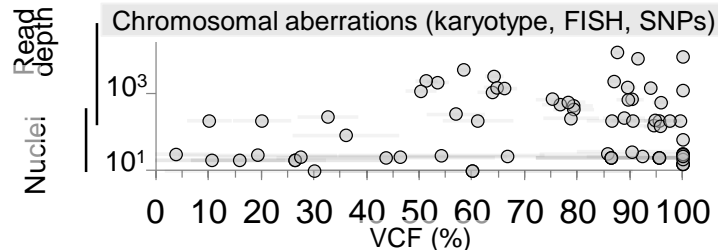


Variant Allele Frequencies (VAF)

Prise en compte des fréquences d'allèle mutés

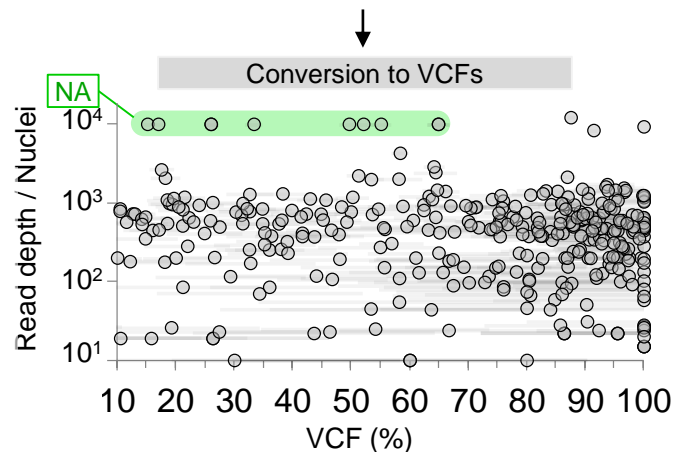


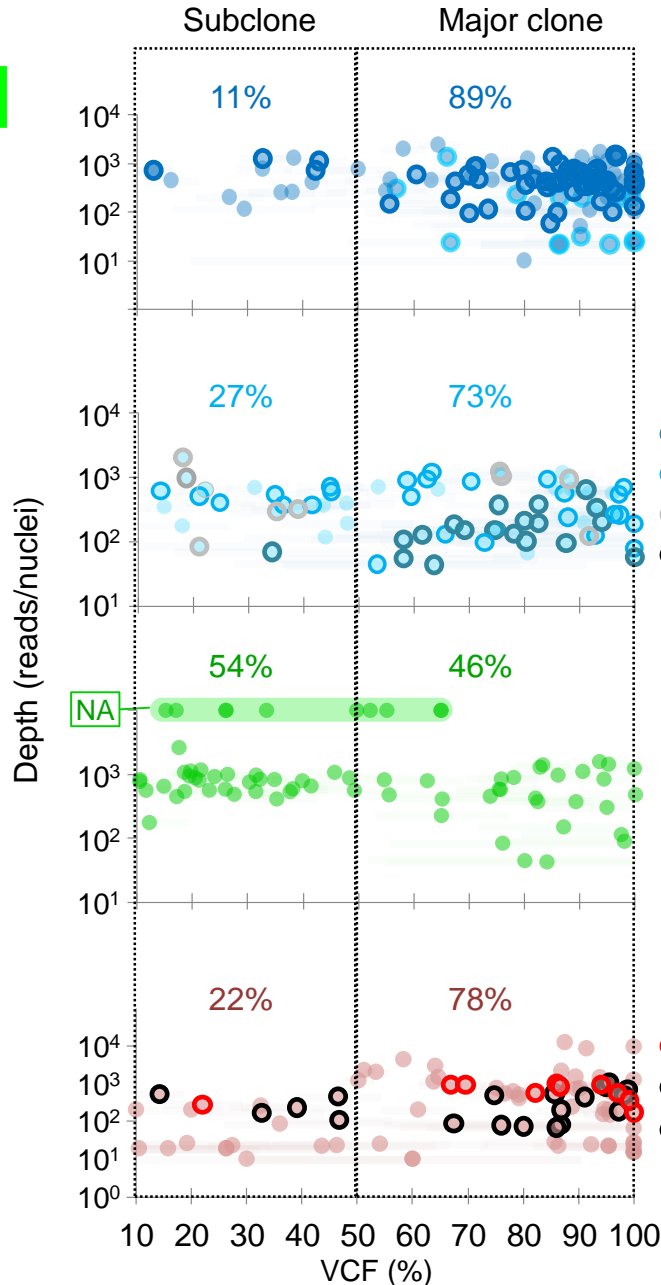
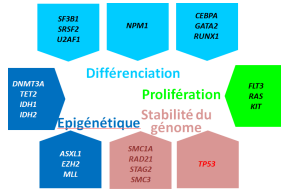
Intégration des données de FISH et caryotype



Variant Cell Frequencies (VCF)

Extrapolation du nombre de cellules porteuses de chaque lésion (VCF)





## Lesions in epigenetic regulators

- CBF and *MLL* rearrangements, del(20q)
- Mutations in *DNMT3A*, *TET2*, *ASXL1*
- Mutations in other epigenetic modifiers

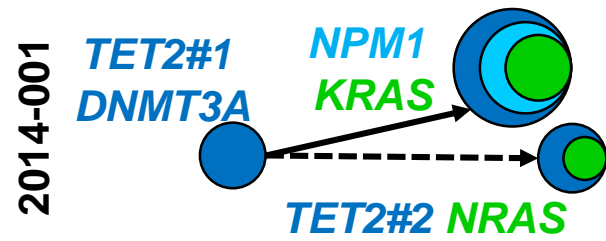
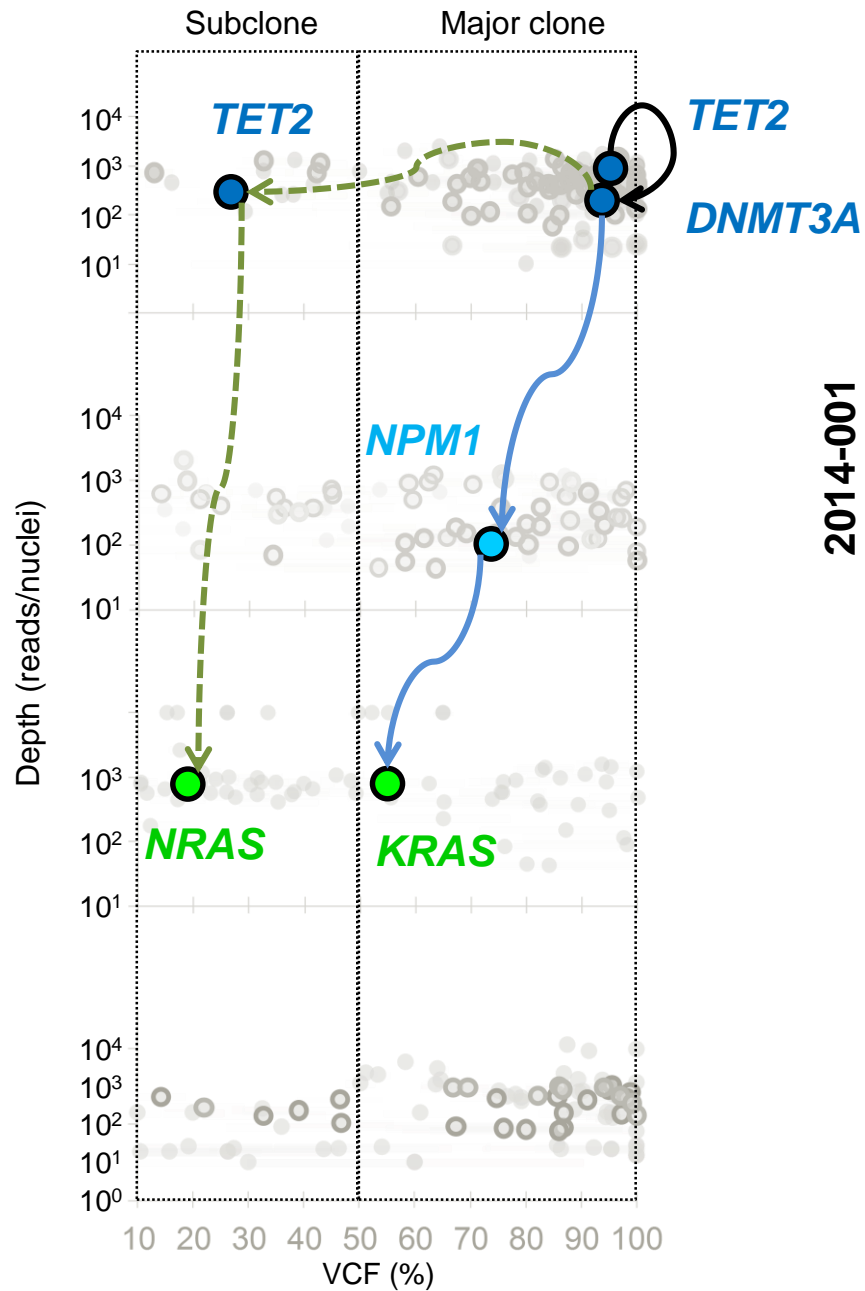
## Mutations in *NPM1*, transcription factors, splicing factors

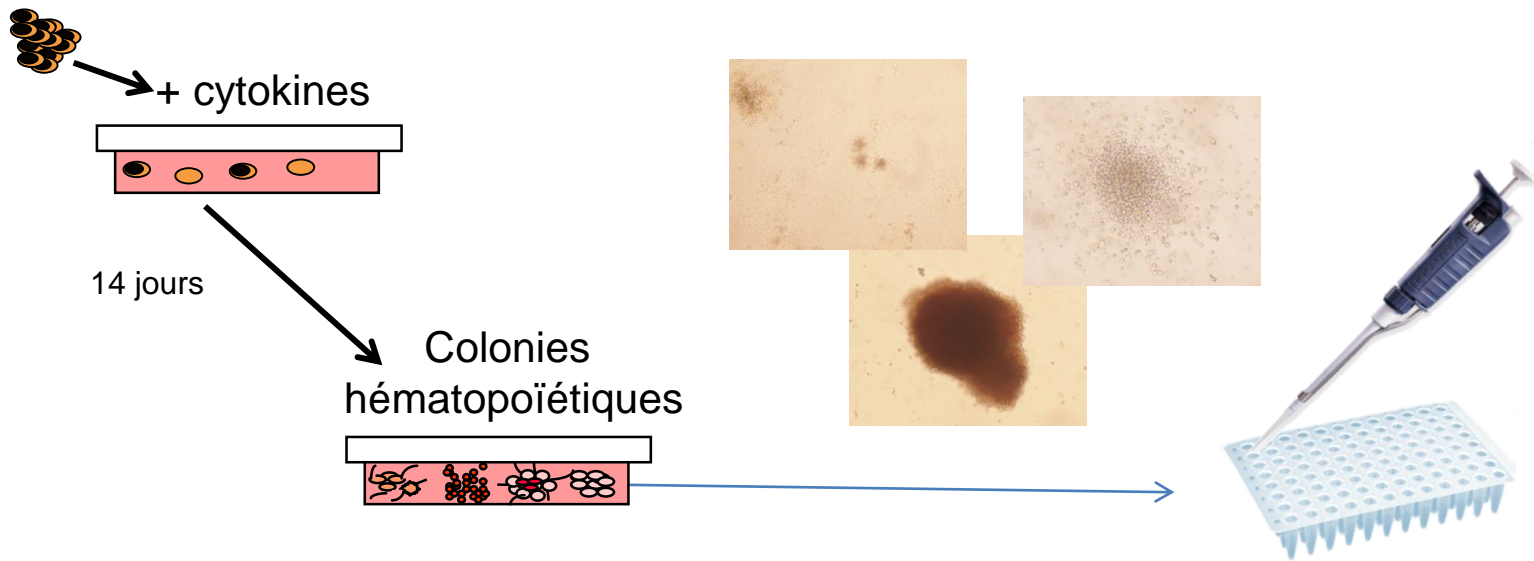
- Mutations in *NPM1*
- Mutations in *RUNX1*, *GATA2*, *CEBPA*
- Mutations in other transcription factors
- Mutations in splicing factors

## Mutations in proliferation/signalling pathways FLT3 RAS KIT

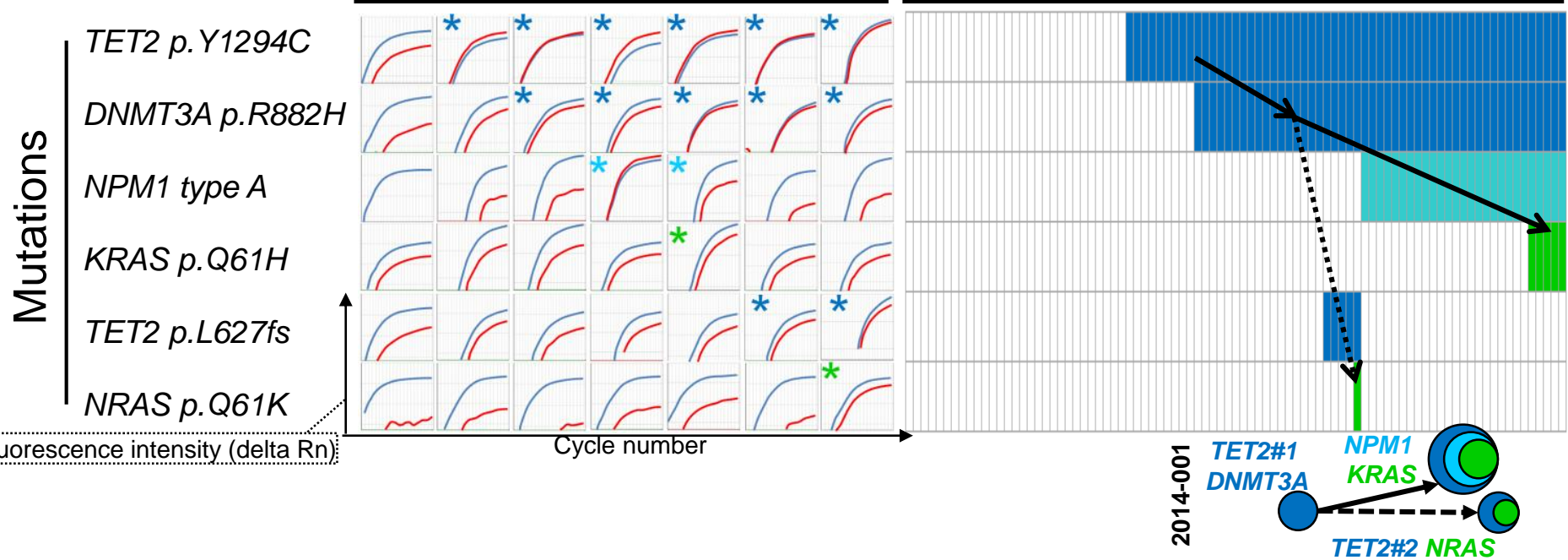
## Other lesions

- *TP53* mutations
- Other chromosomal aberrations
- Other mutations

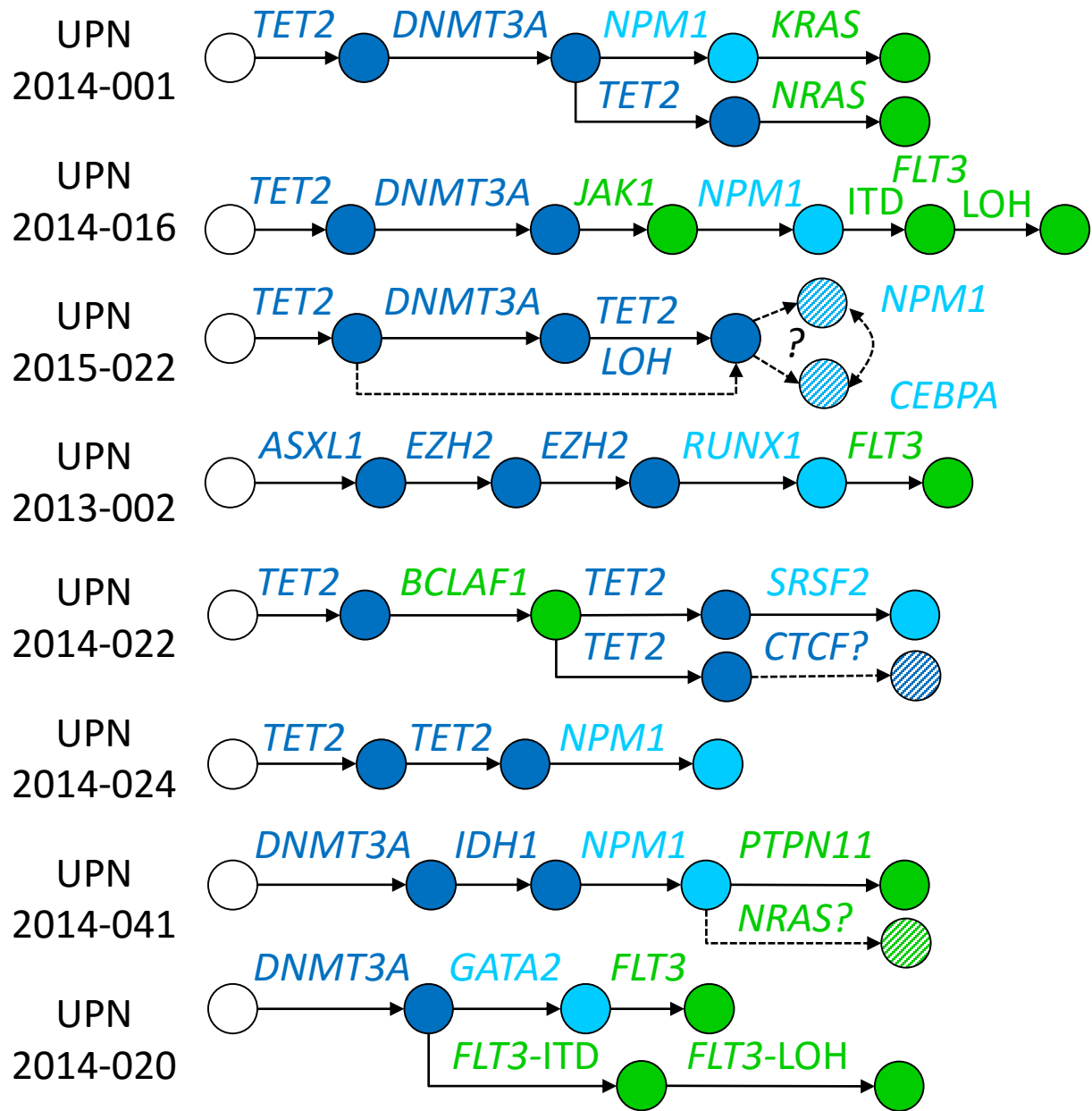




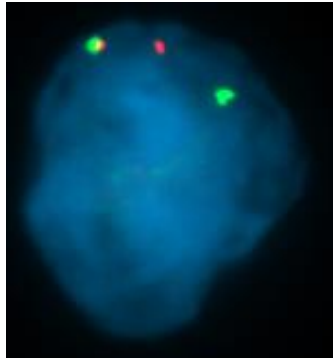
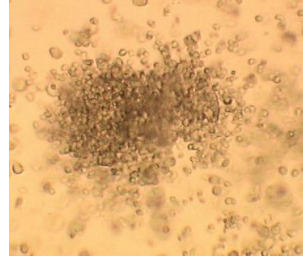
### Colonies



LAM  
Caryotype  
normal

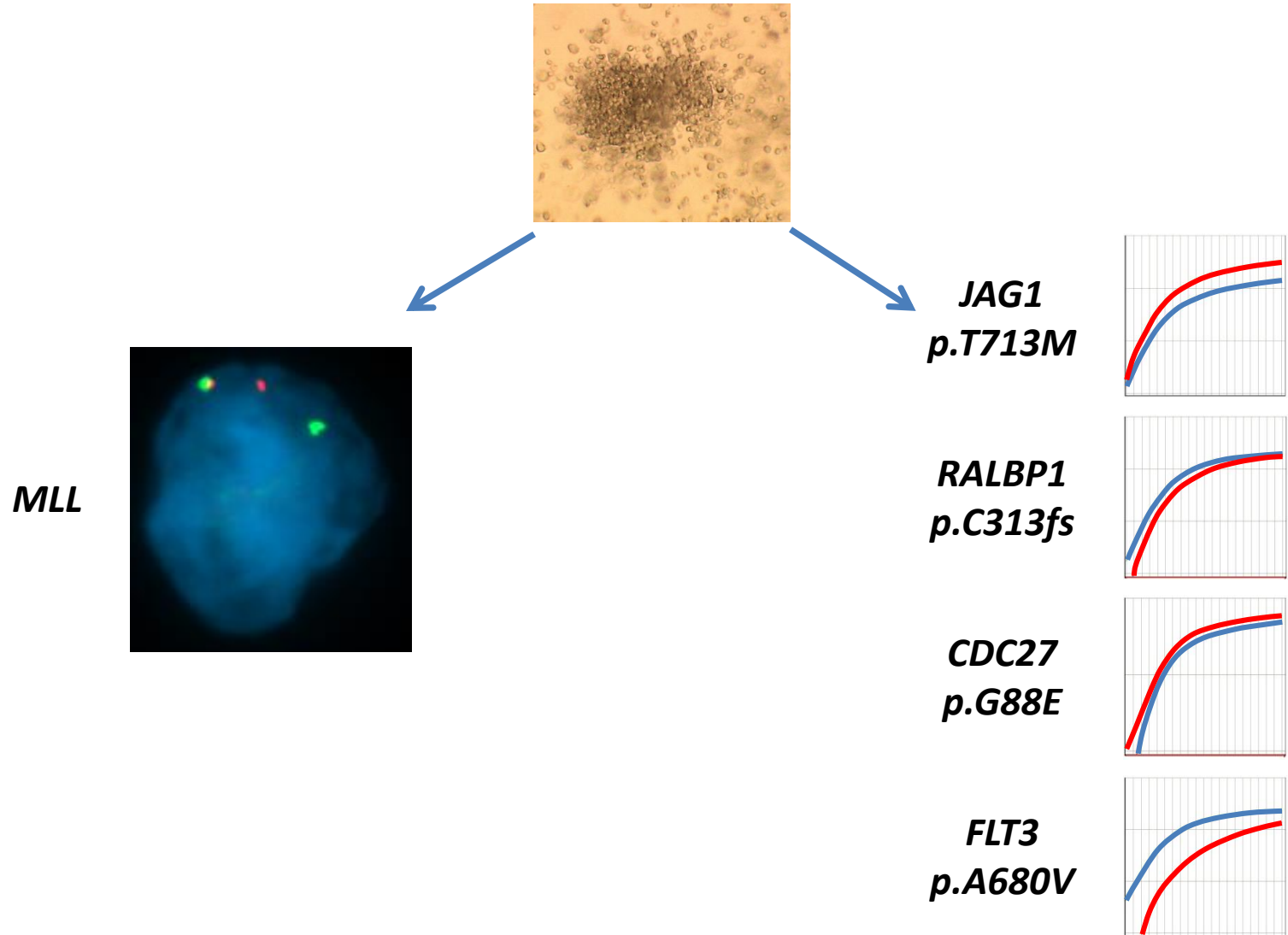


# Génotypage et FISH sur colonies



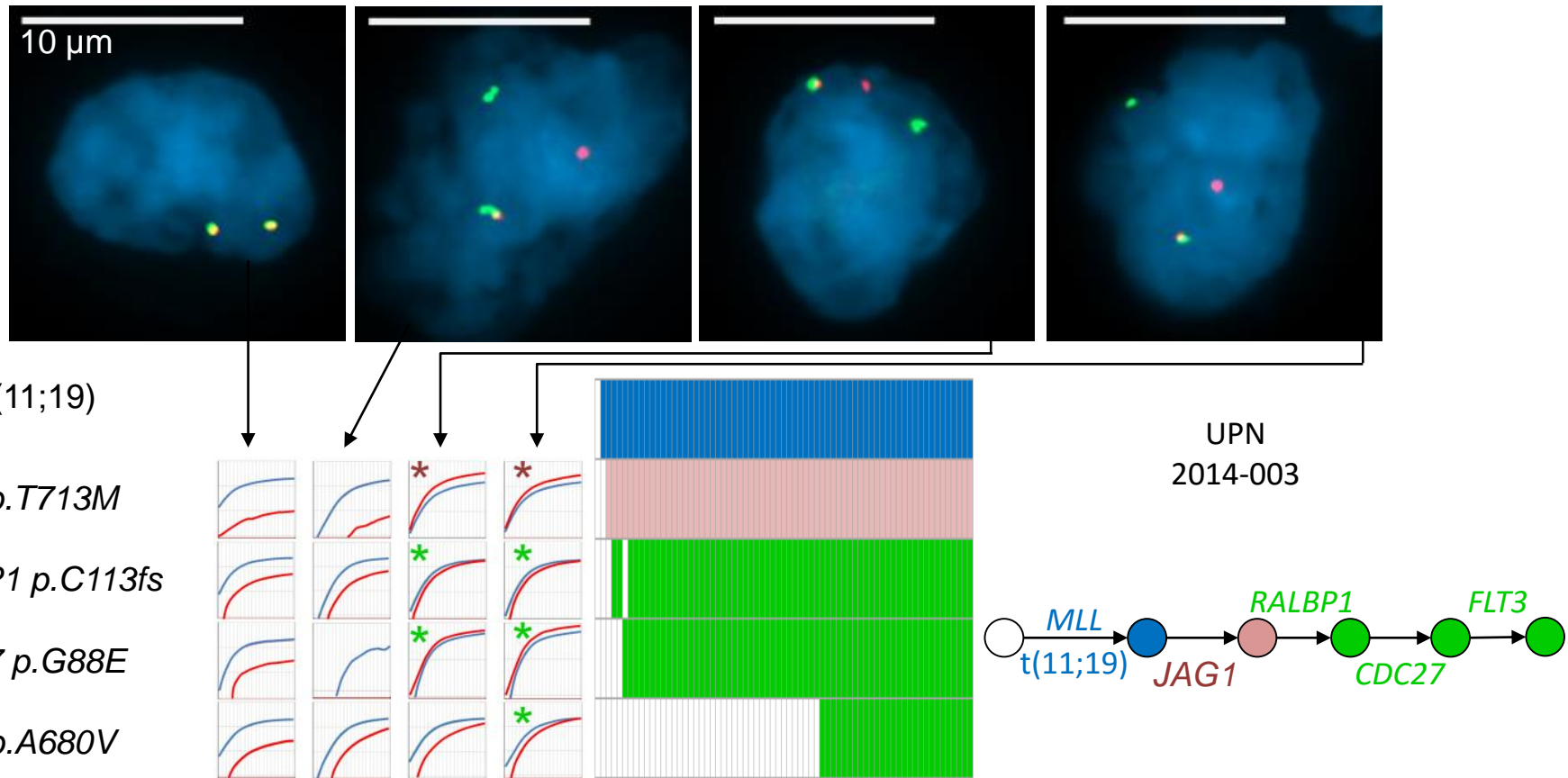
*MLL*

# Génotypage et FISH sur colonies





# Génotypage et FISH sur colonies

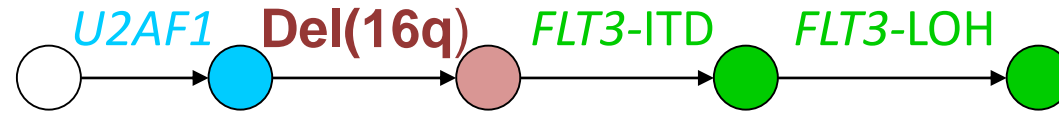


# LAM caryotype anormal

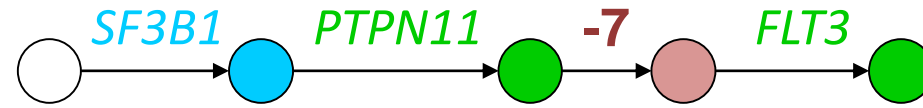
UPN  
2014-015



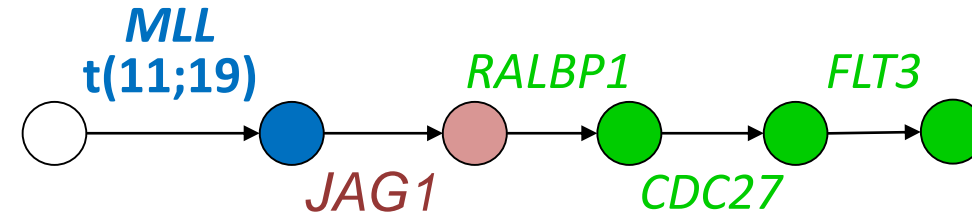
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2014-008



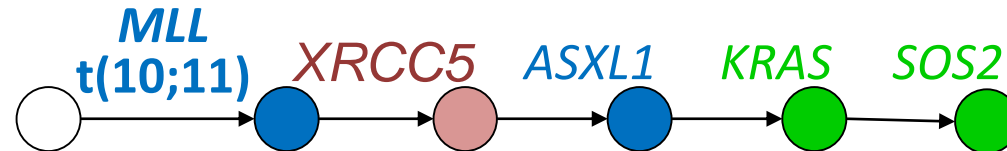
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2014-009



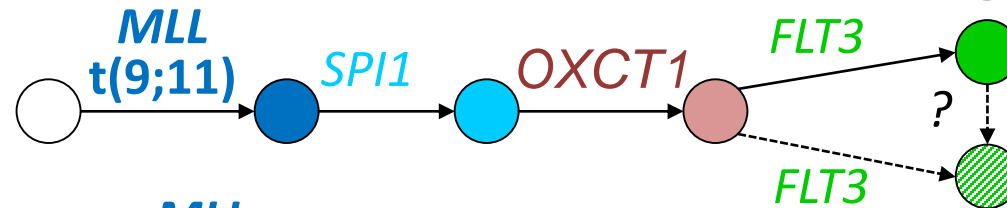
UPN  
2014-003



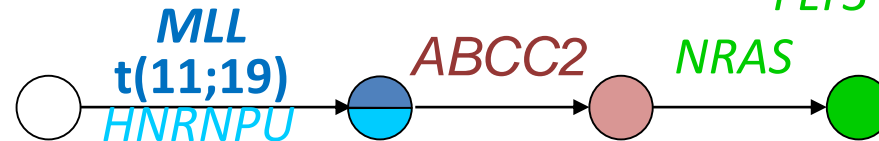
UPN  
2013-004



UPN  
2014-019

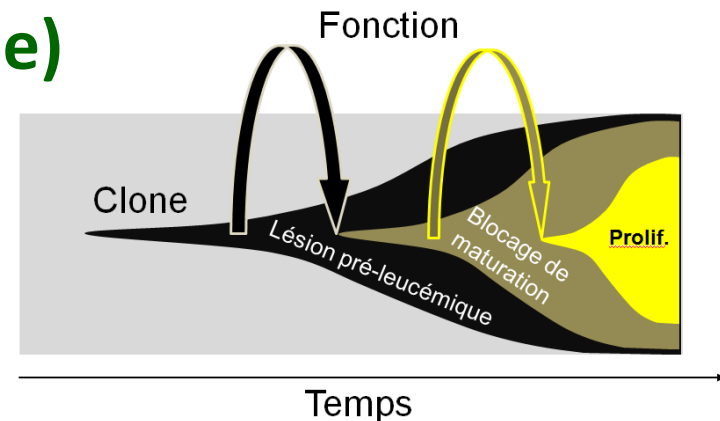


UPN  
2013-001



# Conclusion 1

- Un ordre récurrent
  - **Epigénétique**
  - **Facteurs de transcription/épissage/NPM1c**
  - **Mutations de prolifération (FLT3/RAS/kinases/Cycle)**



- Quelques patients ne répondent pas à cette hiérarchie

# Clone pré-leucémique : deuxième question

- Premières lésions acquises par le clone: parfois des années avant le diagnostic de LAM

## → Ordre d'acquisition des événements: phylogénie clonale

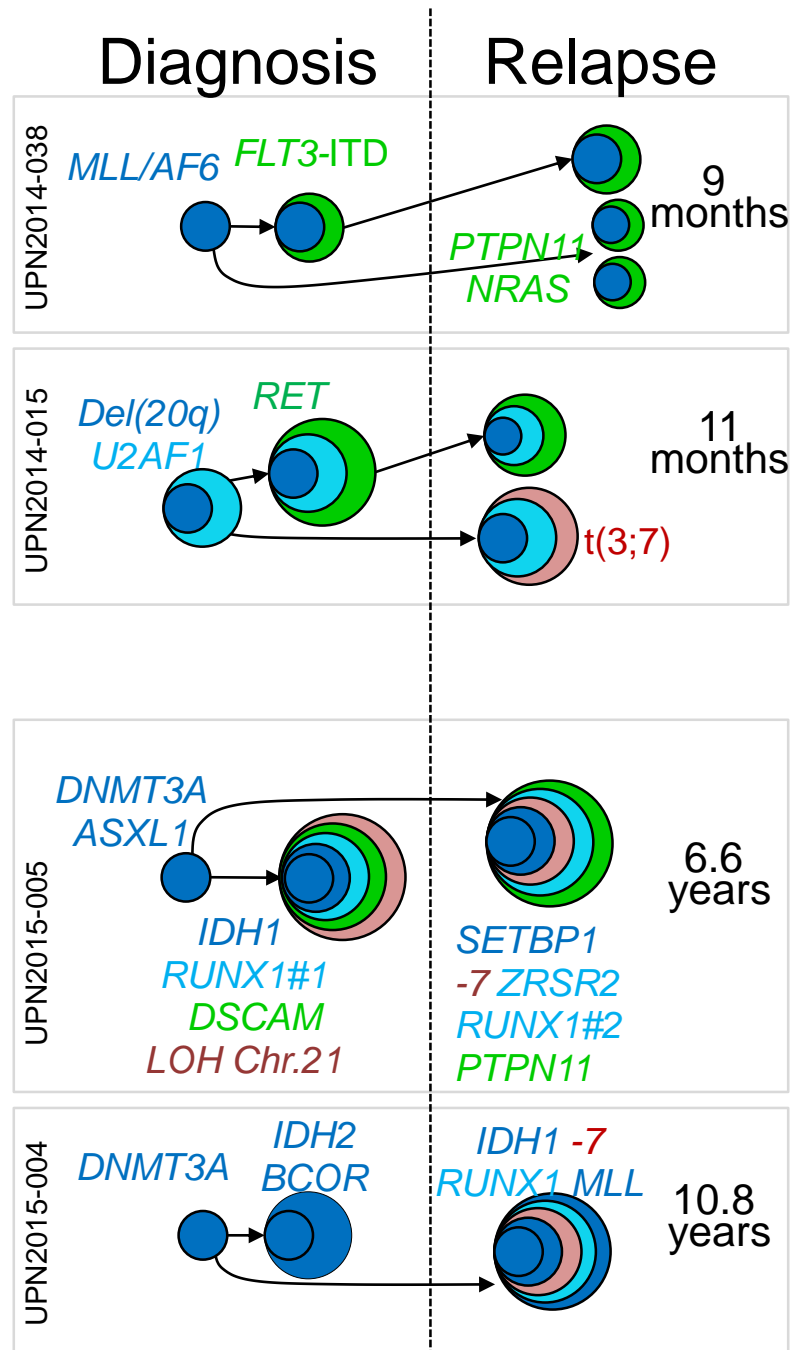
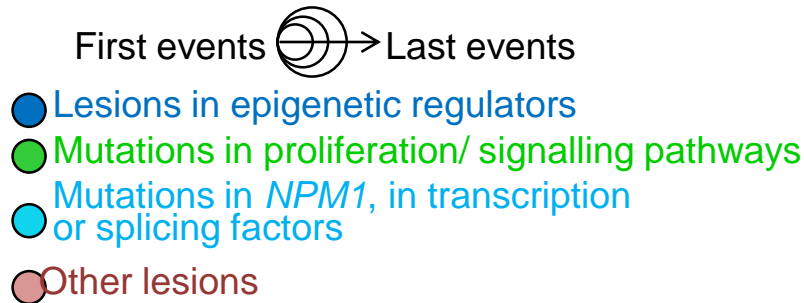
- Lésions capables de persister en rémission complète,  
→ réservoir de la rechute

## → Comparaison composition clonale : diag/RC/rechute

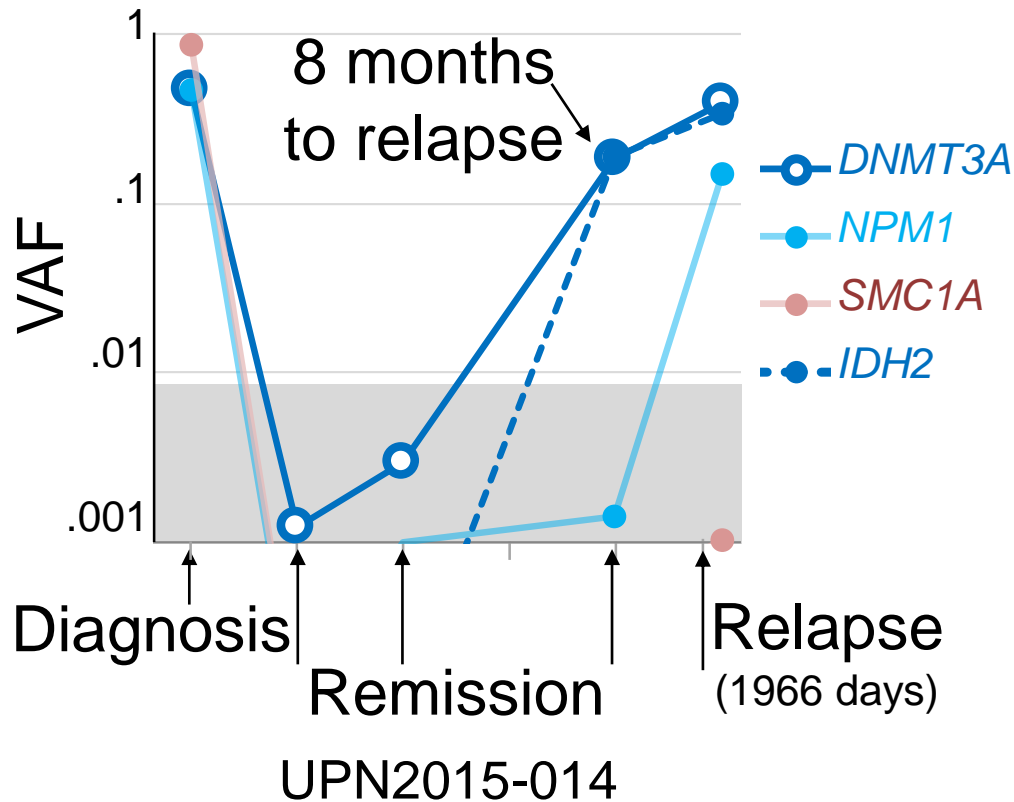
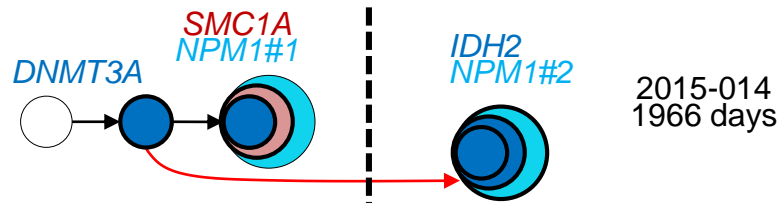
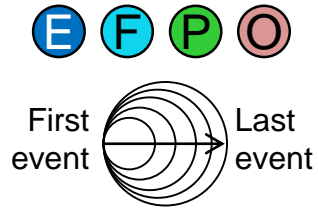
approche intégrée:

- cytogénétique conventionnelle et FISH
- bio mol conventionnelle et NGS (deep seq)
- analyse des colonies

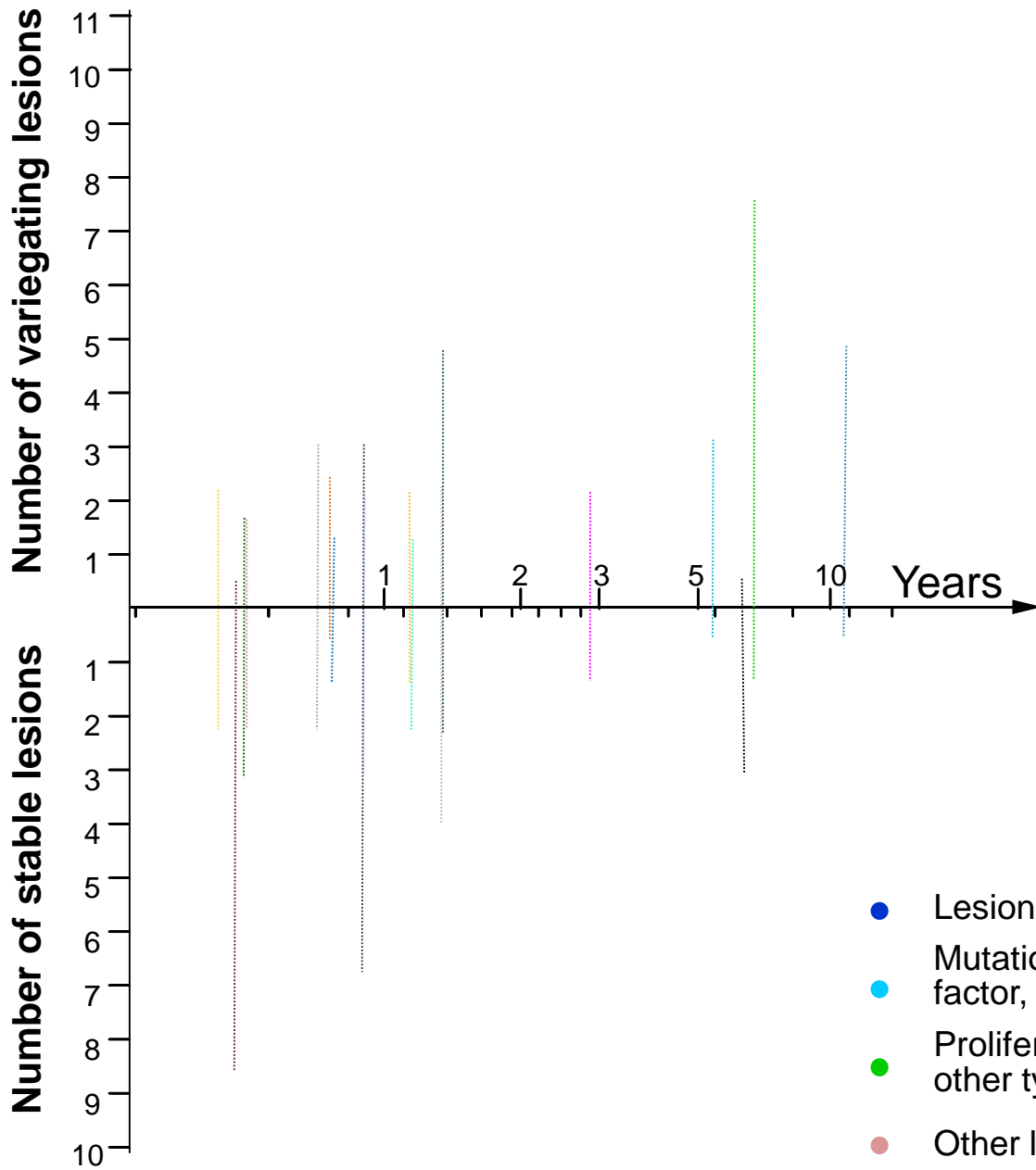
# Comparison : composition clonale: diagnostic vs rechute



# Comparison : composition clonale: diagnostic vs rechute







Time to relapse

- Lesion involving an epigenetic regulator
- Mutation in *NPM1*, in a transcription factor, or in the splicing machinery
- Proliferative mutation (RAS/FLT3 pathways, other tyrosine kinases, cell cycle regulators)
- Other lesions



# Conclusion 2

- Un schéma récurrent
  - Epigénétique: lésions maintenues: *DNMT3A*, *TET2*, *del(20q)*, *MLL*
  - Variégation sur : *NPM1*, évts de prolifération, *IDH+++*
- Rechute tardive = beaucoup de changements sur un clone fondateur stable, ou leucémie secondaire (tout change...)

# Clone pré-leucémique : troisième question

- Premières lésions acquises par le clone: parfois des années (décennies?) avant le diagnostic de LAM

→ **Ordre d'acquisition des événements: phylogénie clonale**

- Lésions capables de persister en rémission complète, constituant le réservoir de la rechute quand celle-ci survient

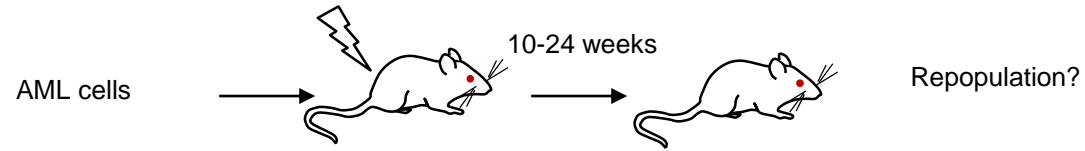
→ **Comparaison composition clonale : diag/RC/rechute**

- Lésions supposées donner un avantage sélectif aux CSH mutées par rapport aux cellules souches non mutées (in vivo: xénogreffes)

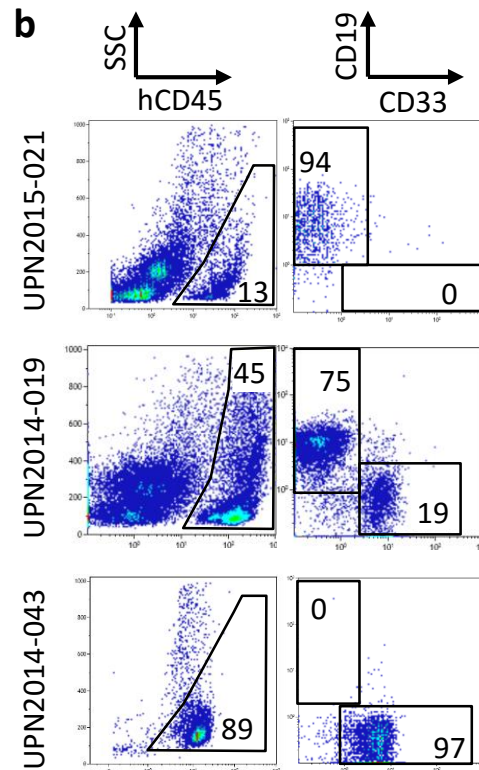
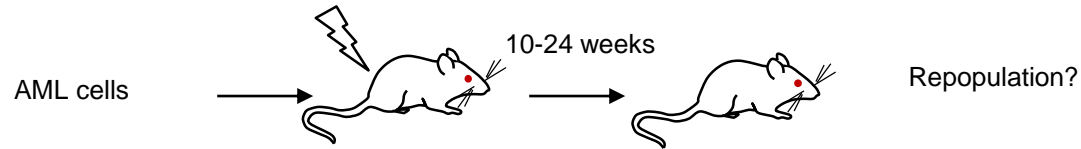
→ **Composition clonale dans les tests in vivo**

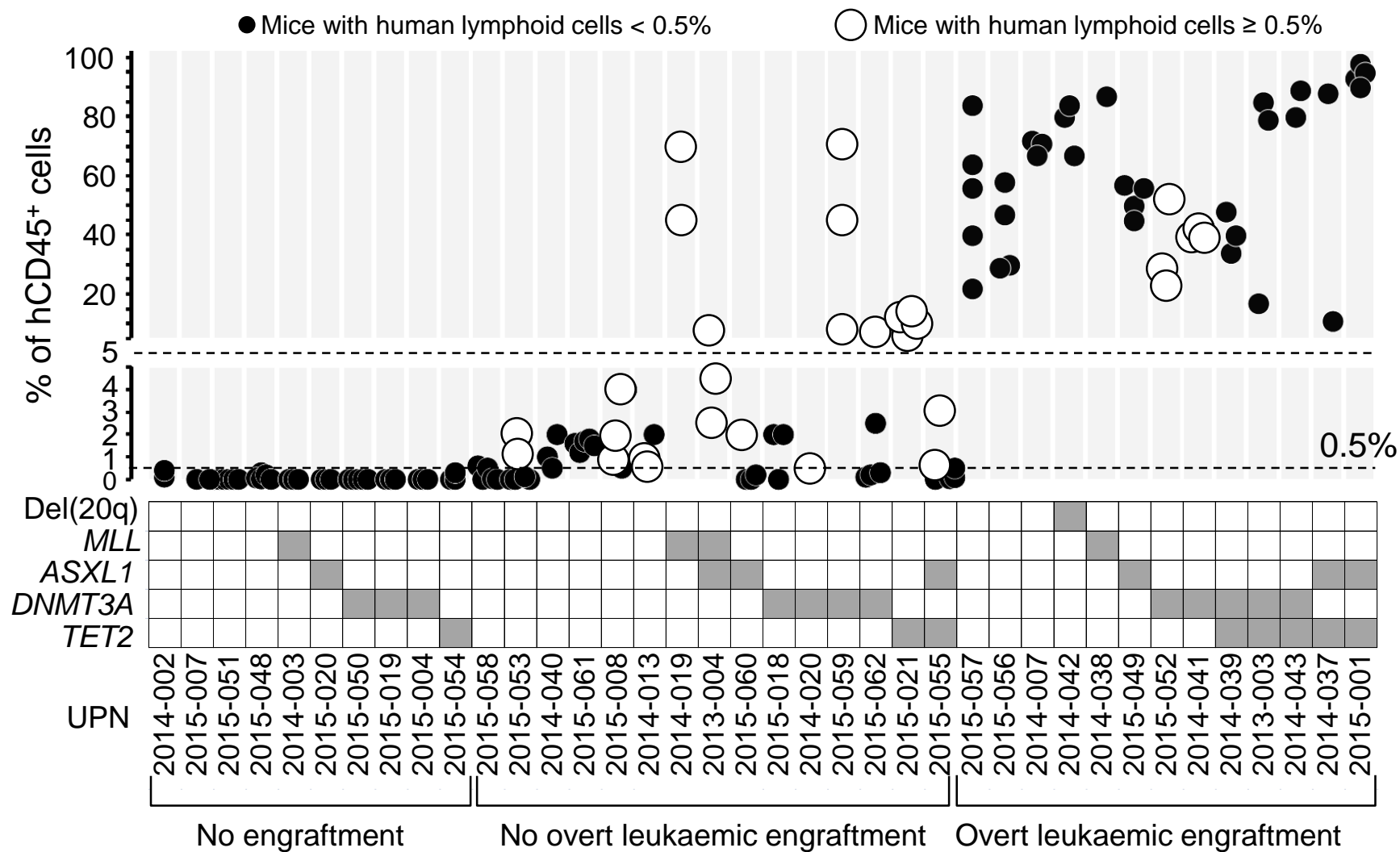
**Modèle Nod/Scid/ $\gamma$ 2a<sup>null</sup> NSG**

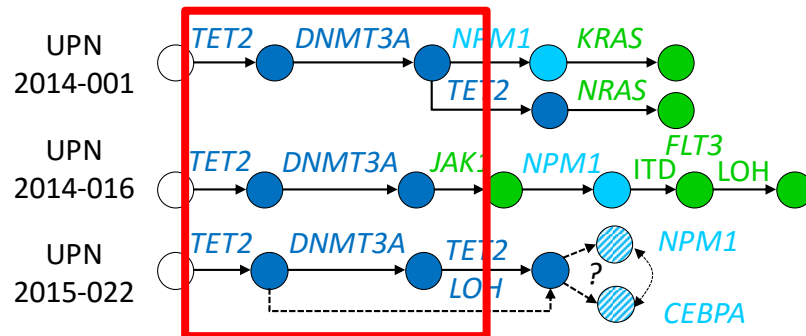
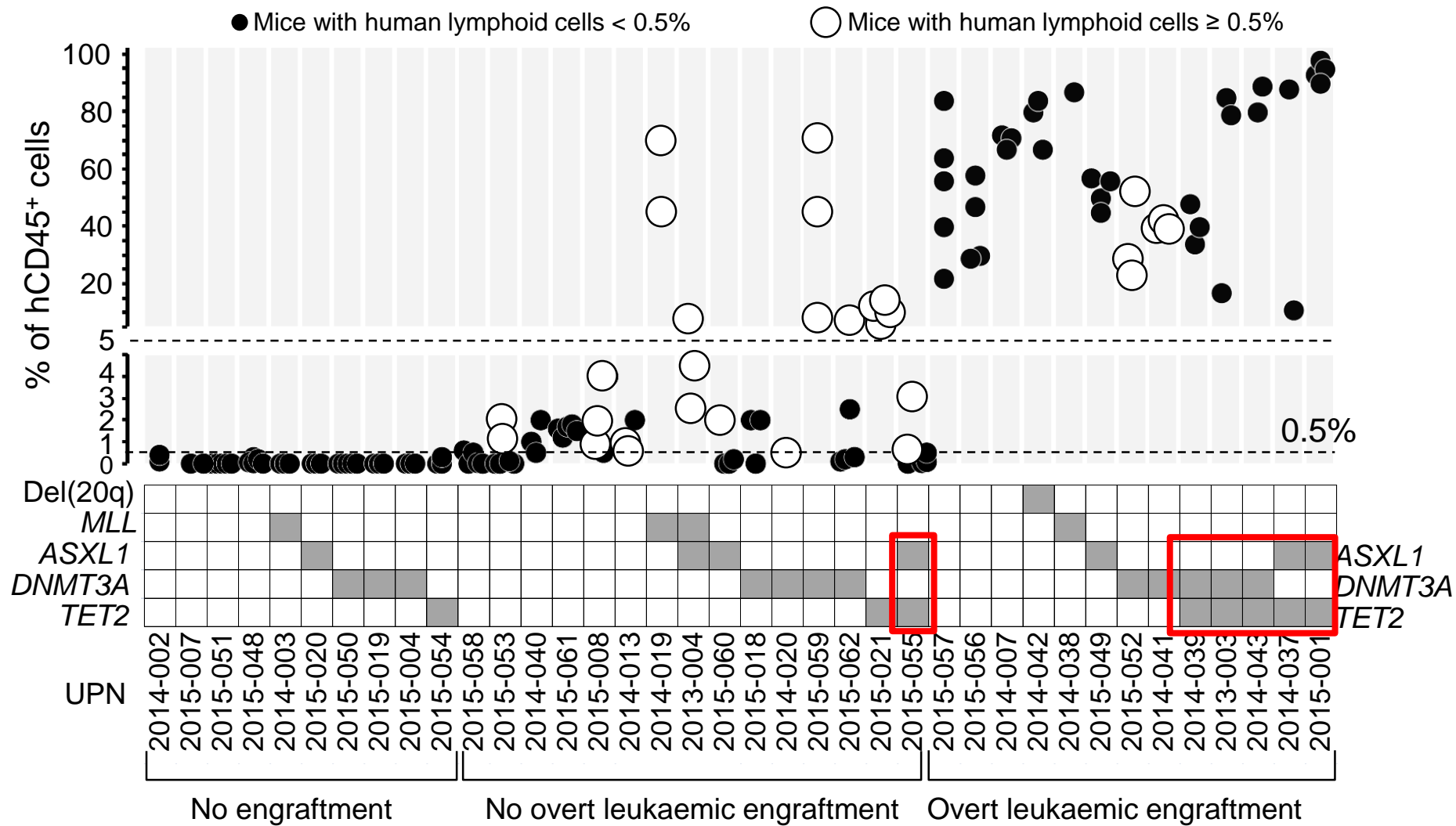
# Xénogreffe

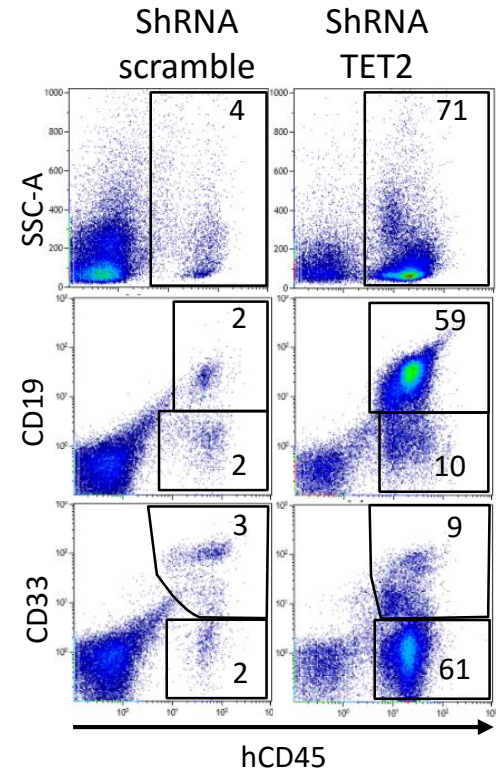
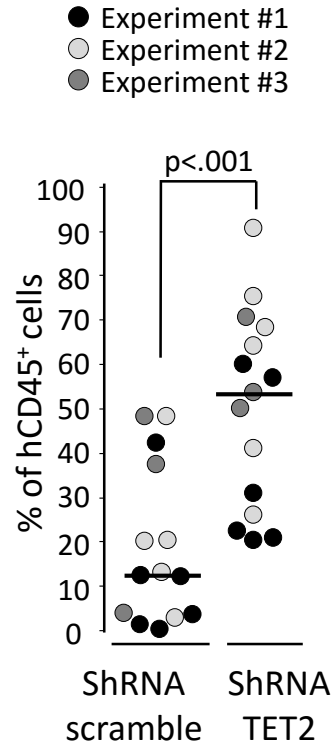
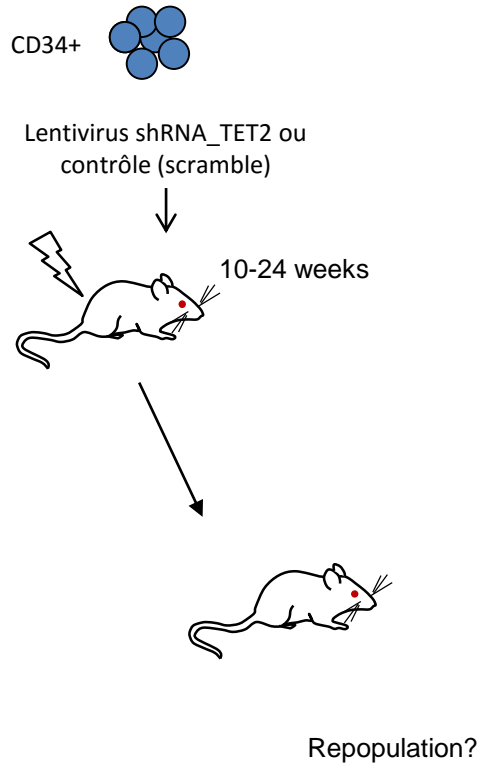


# Xénogreffe





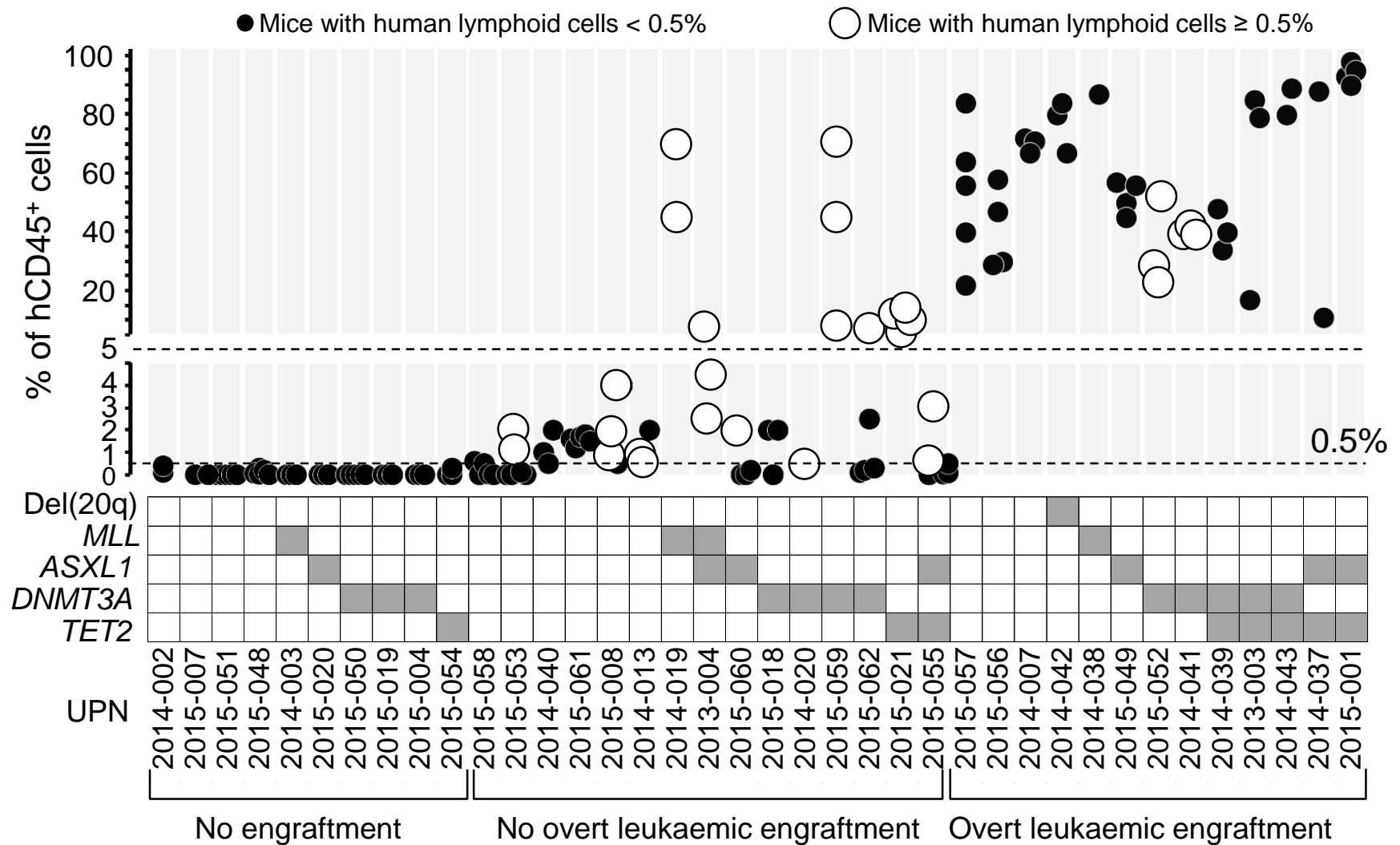




## Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia

Liran I. Shlush<sup>1\*</sup>, Sasan Zandi<sup>1\*</sup>, Amanda Mitchell<sup>1</sup>, Weihsu Claire Chen<sup>1</sup>, Joseph M. Brandwein<sup>1,2,3</sup>, Vikas Gupta<sup>1,2,3</sup>,

**Cellules TET2 KD sont aussi pré-leucémiques**



**Même hypothèse pour toutes les lésions pré-leucémiques? →**  
**DNMT3A TET2, ASXL1, MLL, CBF :** Jan *et al.* *Science Trans Med.* (2012),  
 Bäsecke *et al.* *Leuk. Lymphoma* (2005), Wunderlich, *et al.* *Blood* (2006).



# Conclusion 3

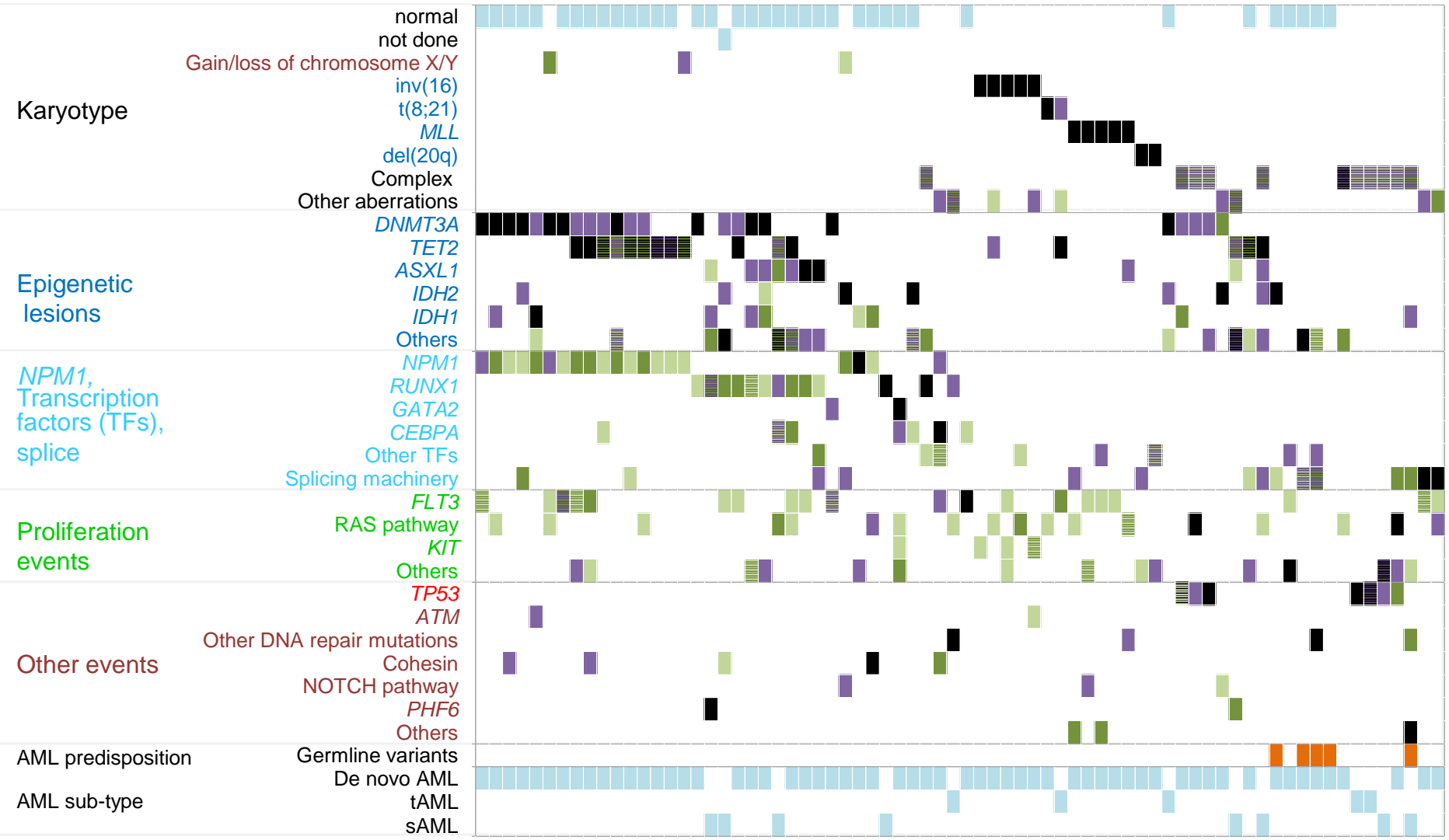
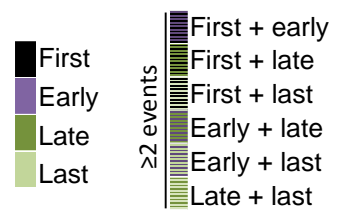
- La prise non leucémique existe pour les LAM  
***DNMT3A, TET2, ASXL1, MLL, CBF***  
et pour certaines autres...

→ la majorité des lésions précoces des LAM entre dans la définition de lésions pré-leucémiques (du modèle de xénogreffe)

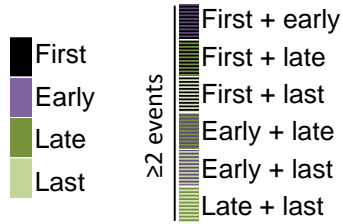
# Implication

Une hiérarchie génétique et fonctionnelle ?

# Order of lesions

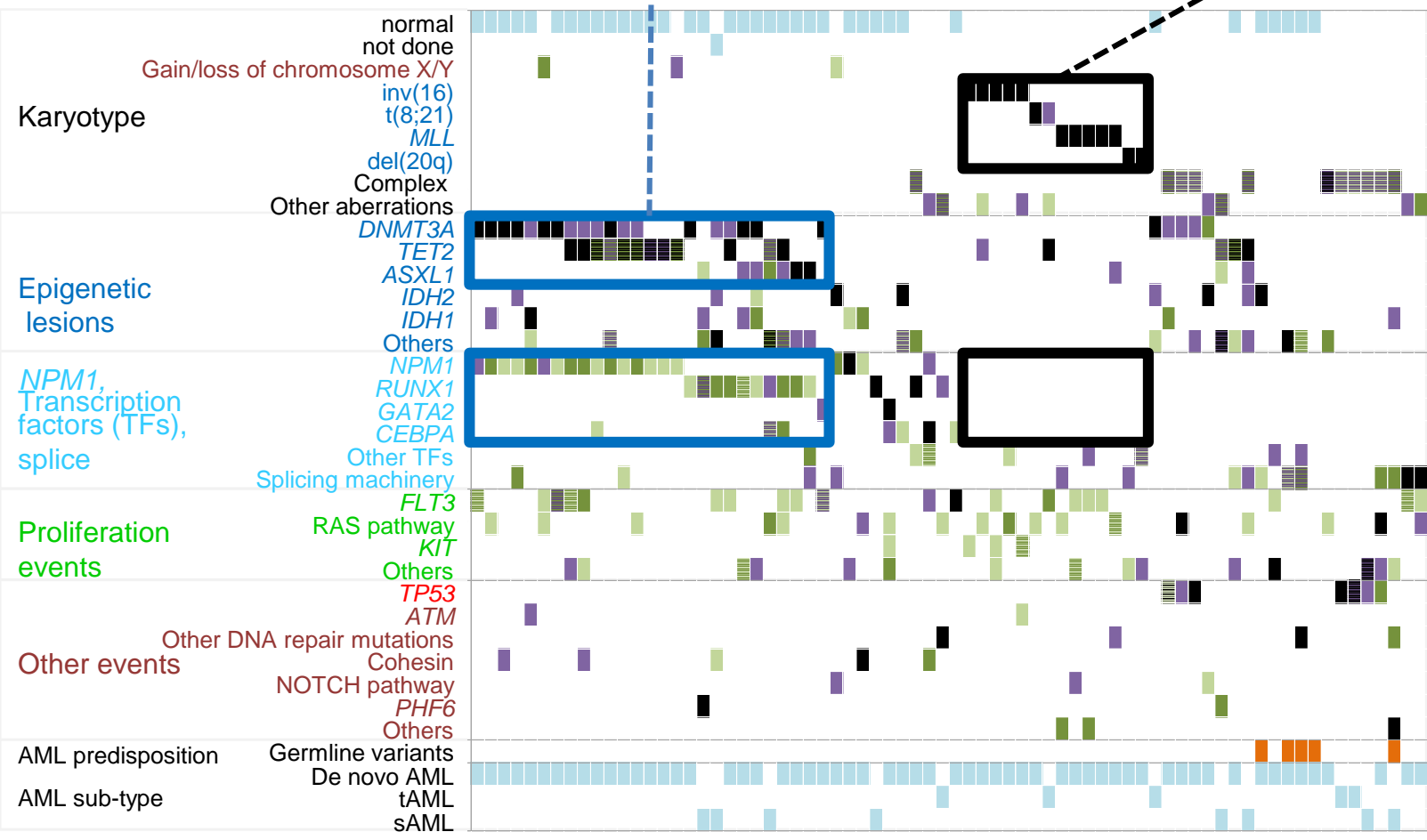


# Order of lesions

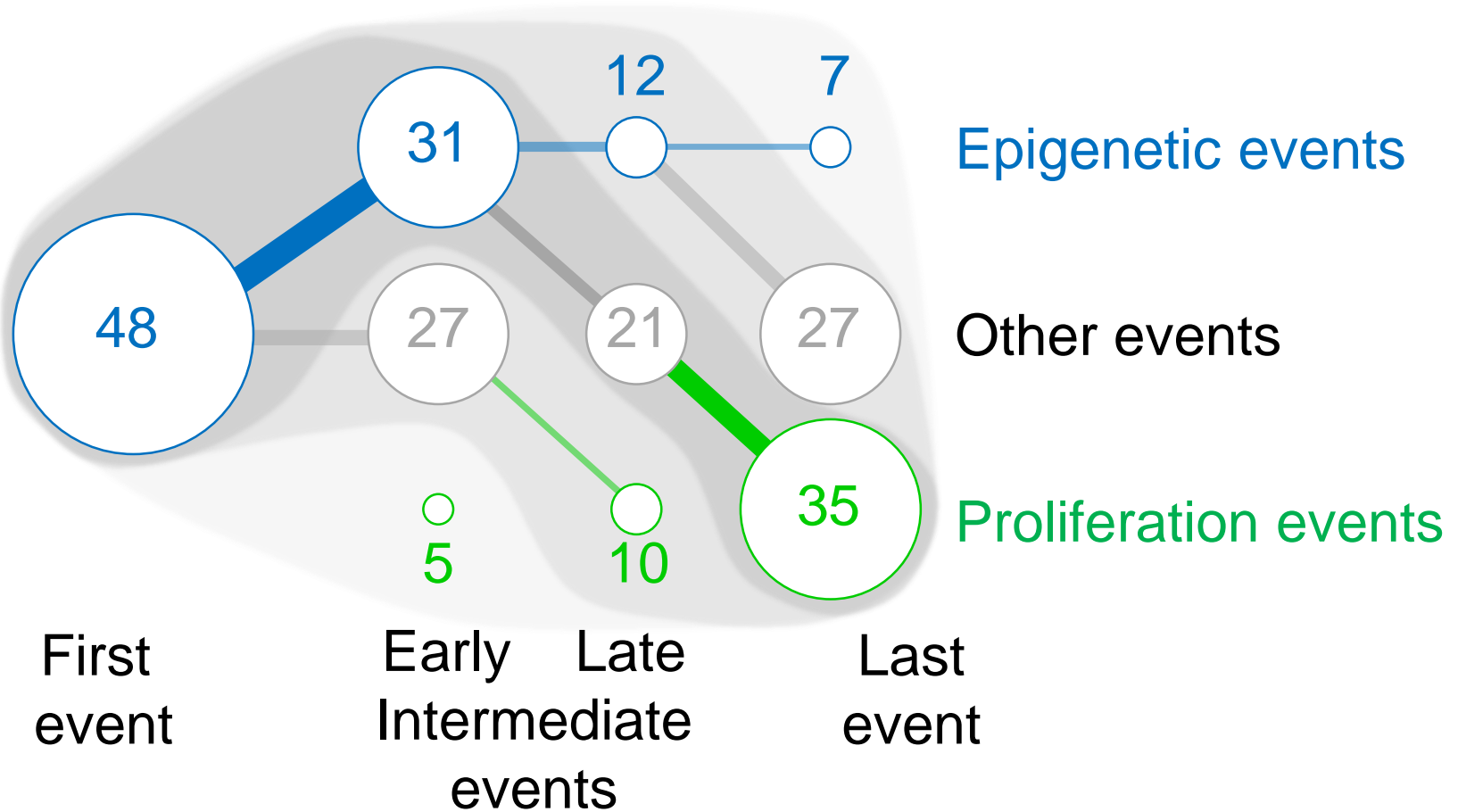


Mutations in master CHIP genes (*DNMT3A*, *TET2*, *ASXL1*)  
 Mutations in *NPM1*, *RUNX1*, *GATA2*, *CEBPA*

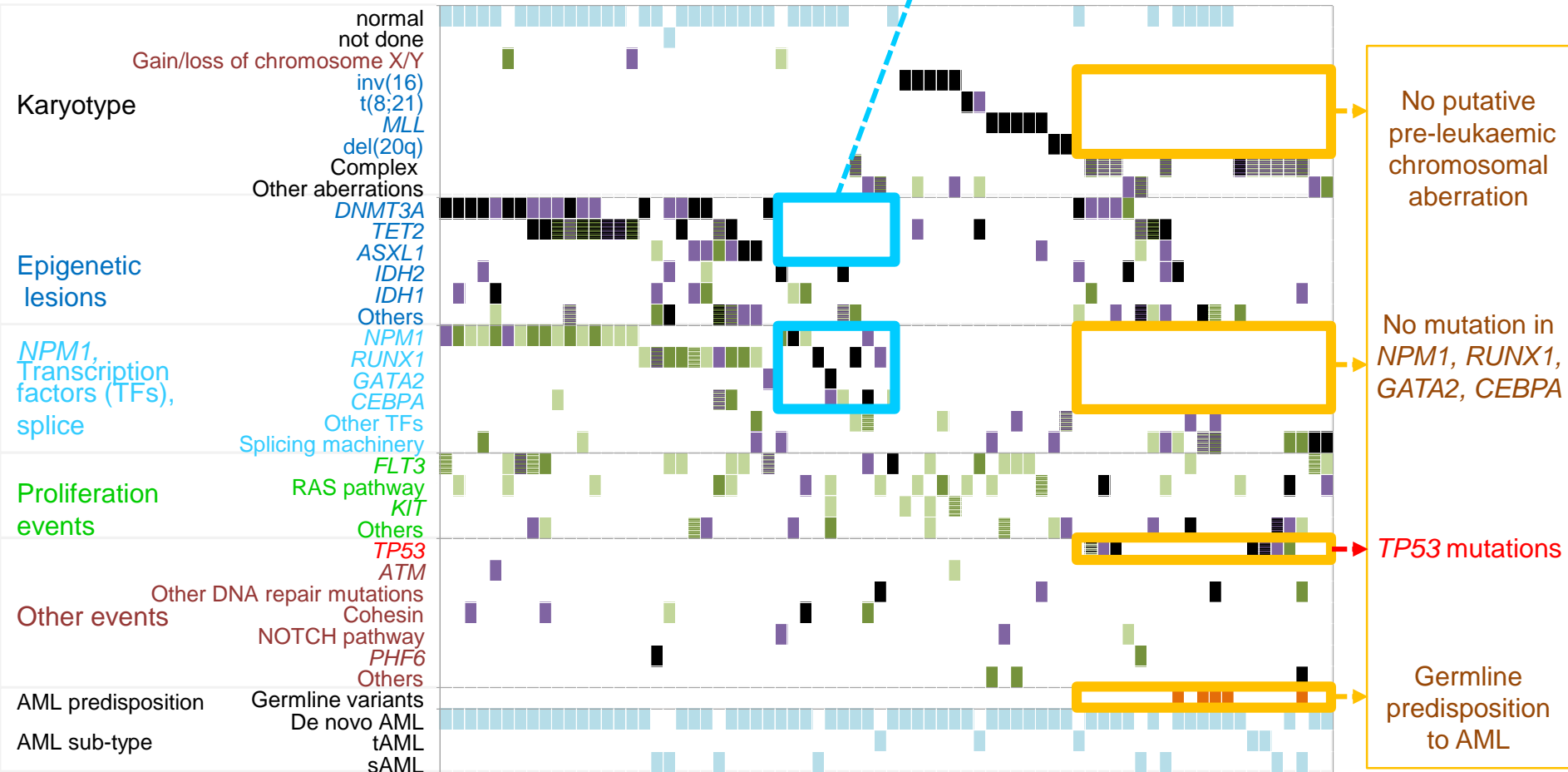
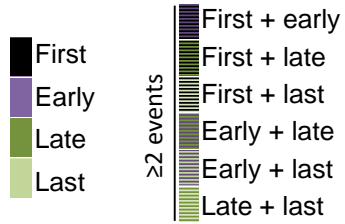
Pre-leukaemic chromosomal aberrations  
 No mutation in *NPM1*, *RUNX1*, *GATA2*, *CEBPA*



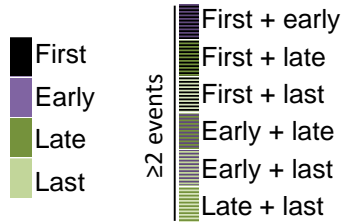
# Lésions d'expansion pré-leucémique



# Order of lesions



# Order of lesions



**GROUP 1**

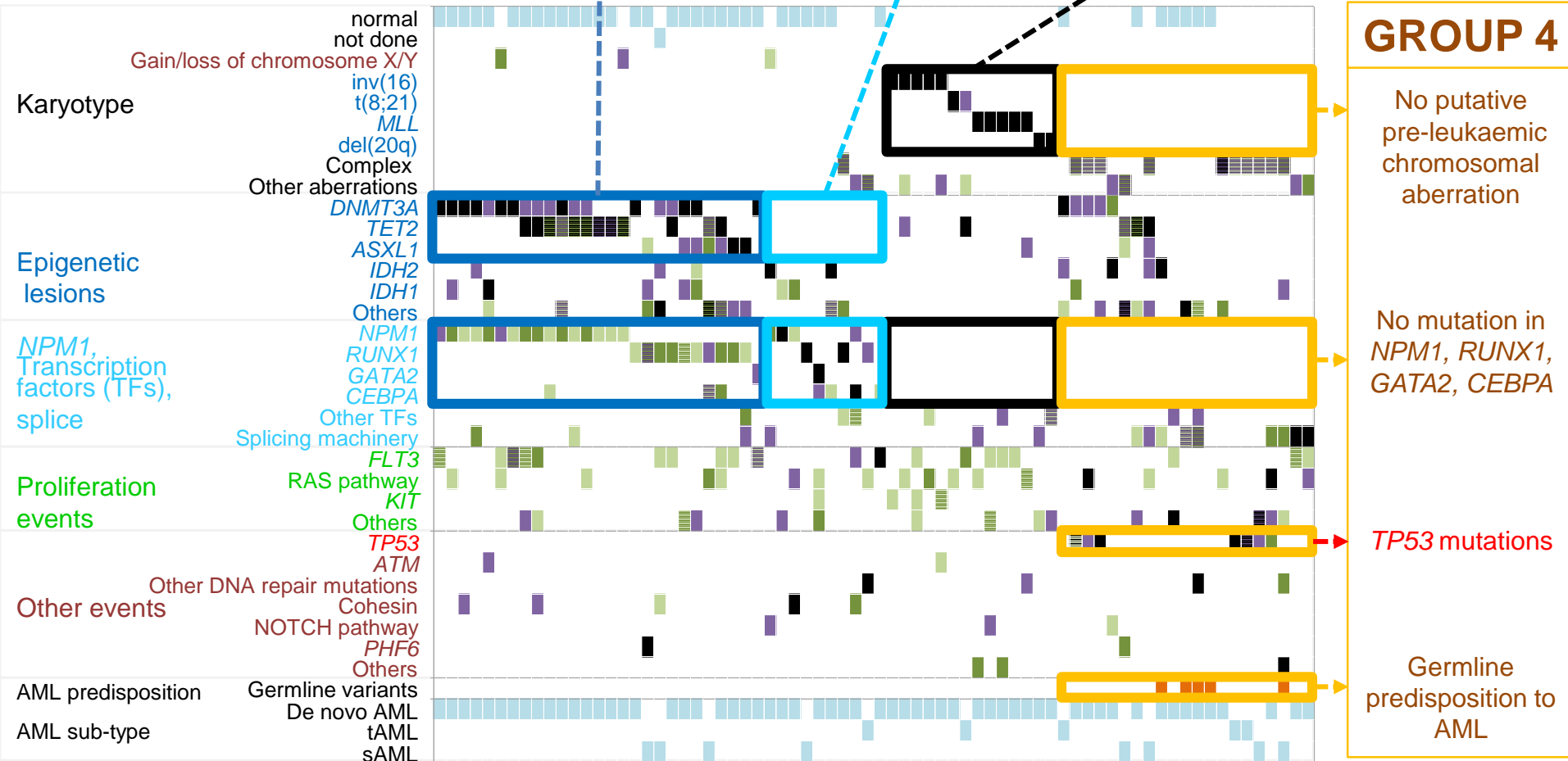
Mutations in master ARCH genes (*DNMT3A*, *TET2*, *ASXL1*)  
 Mutations in *NPM1*, *RUNX1*, *GATA2*, *CEBPA*

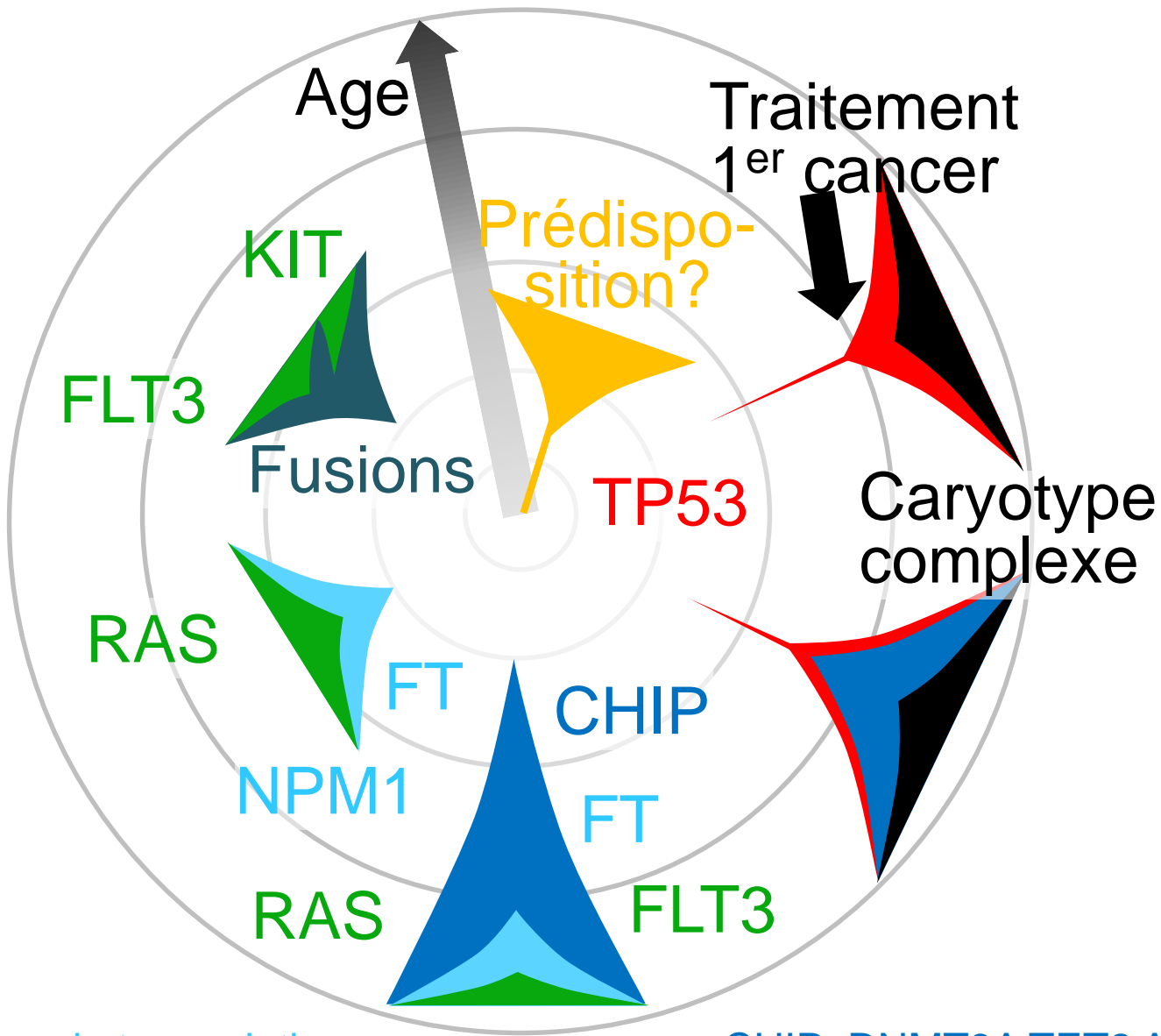
**GROUP 2**

No master ARCH mutation,  
 Mutations in *NPM1*, *RUNX1*, *GATA2*, *CEBPA*

**GROUP 3**

Pre-leukaemic chromosomal aberrations  
 No mutation in *NPM1*, *RUNX1*, *GATA2*, *CEBPA*





FT: facteurs de transcription

CHIP: DNMT3A TET2 ASXL1



# Conclusions et perspectives : LAM

-> **Hiérarchie génétique et temporelle**

-> Le **lien fonctionnel** reste peu étudié:

→ pourquoi CBF -> KIT, DNMT3 -> NPM1, ASXL1 -> RUNX1??

-> Profils suggérant des **prédispositions**: LAM adulte/enfant:

état préleucémique « acquis / constitutionnel »

-> L'architecture clonale: un outil pour le suivi « **clonospécifique** »  
de la **maladie résiduelle**