Hydroxyurea-Driven *TP53* homozygosity accelerates Leukemic Transformation in *CALR*-mutant MPNs

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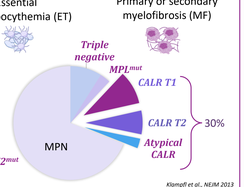
***SB and MB equally contributed ; #IP and GH co-lead the work**

INTRODUCTION

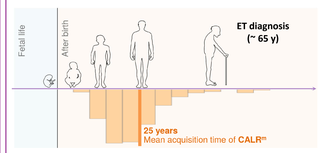
Classical myeloproliferative neoplasms (MPN) result from the acquisition of driver mutations in hematopoietic stem cells (HSCs). One driver mutation affects the *CALR* gene (*CALR^{mut}*), resulting in constitutive activation of the MPL/JAK2/STAT pathway. A severe complication of these diseases is their transformation into secondary acute myeloid leukemia (AML). This is associated in 30% of the cases with the acquisition of multihit mutations in the gene encoding the transcription factor *TP53*, while this mutation is only present in 7% of the MPN in chronic phase.

Driver Mutation : *CALR^{mut}*



Klampfl et al., NEJM 2013
Chachoua et al., Blood 2016
Araki et al., Blood 2016
Elf et al., Blood 2017
Park et al. PLoS ONE 2015

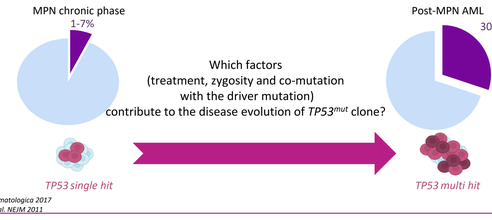
Early *CALR^{mut}* onset



✓ Mutations are acquired in HSC early in life and amplified over life

Hermange et al. PNAS, 2022
Sousos et al. Nat Medicine, 2022

Leukemic transformation is associated with multihit *TP53* mutations



Courtier et al. Haematologica 2017
Harutyunyan A et al. NEJM 2011

OBJECTIVES

Reconstruct the natural history of clone *TP53^{mut}* during MPN development and transformation.

Specific questions :

✓ How fast *TP53* mutated clone expands?

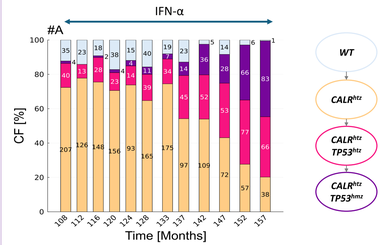
✓ When are the single-hit or multi-hit *TP53* mutations acquired in HSCs?

RESULTS

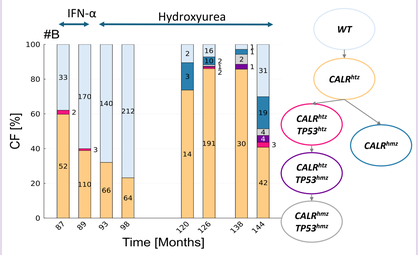
Clonal architecture in progenitors over time

CF= Clonal Fraction = proportion of mutated cells
Numbers within each segment indicate the number of colonies.

Patient #A
CALR^{del34}/TP53^{V143M}



Patient #B
CALR^{del13}/TP53^{D281E}



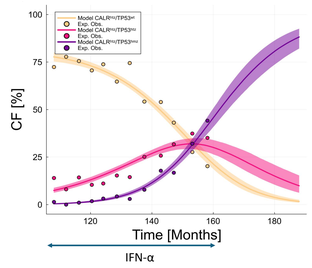
✓ Complex clonal architecture involving *TP53* multihit acquisition

✓ *TP53^{mut}* is acquired in *CALR^{mut}* clone

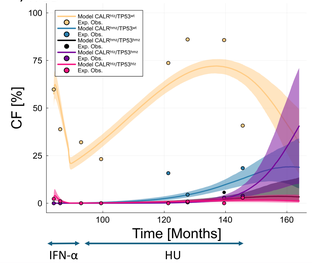
Clonal evolution dynamics within progenitors (HSPC)

✓ Mathematical model-based estimation of the clonal fraction evolution within progenitors

Patient #A
CALR^{del34}/TP53^{V143M}



Patient #B
CALR^{del13}/TP53^{D281E}



Over time, the proportion of *TP53^{multihit}* HSPCs increases more rapidly than that of *TP53^{htz}* HSPCs independently of the type of treatment (IFNα /hydroxyurea (HU)).

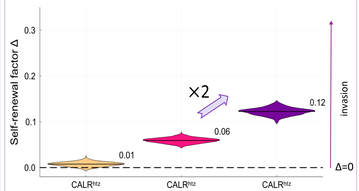
Self-renewal factor Δ of mutated HSCs

Δ= 0 : WT = mut

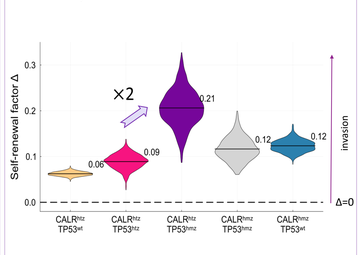
Δ< 0 :

Δ> 0 :

Patient #A
CALR^{del34}/TP53^{V143M}



Patient #B
CALR^{del13} /TP53^{D281E}

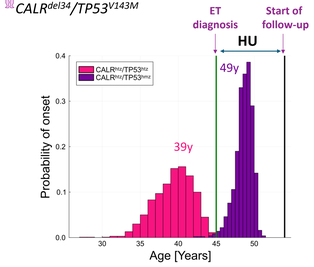


✓ Amplification of all *CALR^{mut}* HSCs

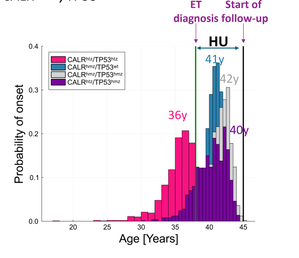
✓ *CALR^{htz} /TP53^{wt} < CALR^{htz}/TP53^{htz}< CALR^{htz}/TP53^{multihit}*

Age of acquisition of *TP53^{htz}* and *TP53^{hmz}*

Patient #A
CALR^{del34}/TP53^{V143M}



Patient #B
CALR^{del13} /TP53^{D281E}



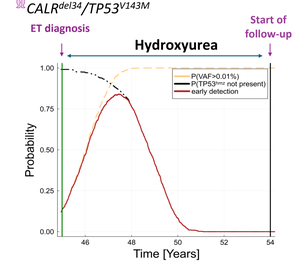
✓ *TP53^{htz}* arose before ET diagnosis with high probability (100% (#A) – 90%(#B))

✓ *TP53^{hmz}* onset during Hydroxyurea therapy (99% – 84%) and 10 years before transformation

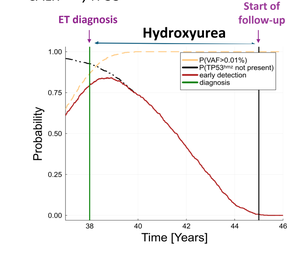
Inference of early strategy to detect *TP53* mutation

✓ Using our calibrated mathematical model to infer past cell dynamics, we assessed whether early detection could have been achieved with a VAF sensitivity of 0.01%

Patient #A
CALR^{del34}/TP53^{V143M}



Patient #B
CALR^{del13}/TP53^{D281E}



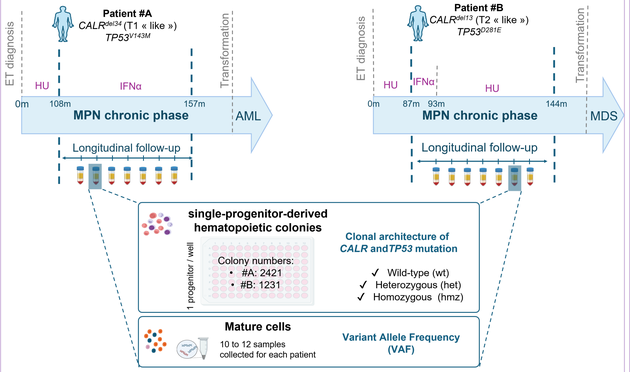
From our mathematical model, we estimate that :

✓ For patient A, *TP53^{htz}* is undetectable at ET diagnosis but reaches ~75% probability of detection within 2 to 3 years.

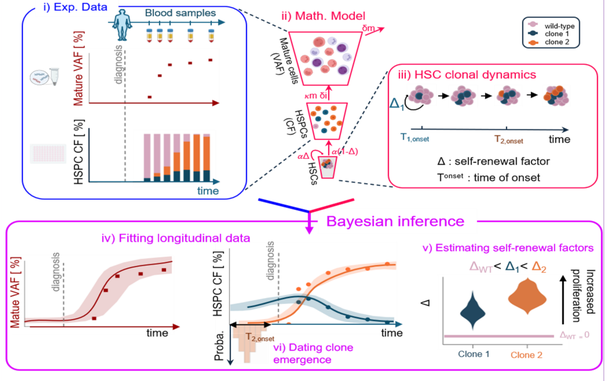
✓ For patient #B, *TP53* mutation is detectable at ET diagnosis

METHODS

① Clonal architecture on single-cell derived colonies



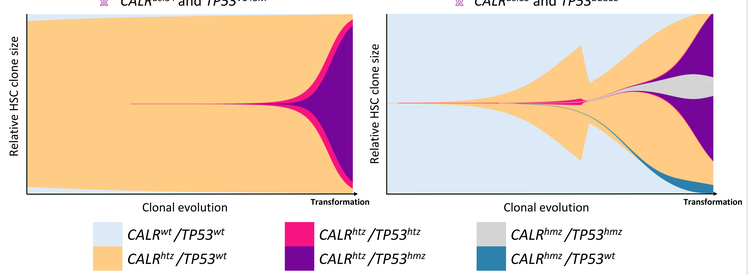
② Inference of cell dynamics with mathematical model



CONCLUSIONS

Patient A with *CALR^{del34}* and *TP53^{V143M}*

Patient B with *CALR^{del13}* and *TP53^{D281E}*



✓ *CALR^{htz}/TP53^{hmz}* HSC proliferate twice as fast as *CALR^{htz}/TP53^{htz}* HSC independently of the type of treatment (Hydroxyurea/IFNα).

✓ Inferred acquisition of *TP53^{htz}* 2 years for patient A and 6 years for patient B before the diagnosis of ET

✓ Inferred acquisition of *TP53^{hmz}* 2 years for patient A and 4 years for patient B after hydroxyurea initiation and 10 years before transformation

PERSPECTIVES

✓ Study the dynamics of *CALR^{mut}/TP53^{mut}* clonal expansion in a larger cohort of patients

✓ Both patients were classified as low-risk MPN at diagnosis, yet this converging high-risk profile highlights the need for closer monitoring and high-sensitivity TP53 NGS in clinical practice.

✓ Repeated monitoring of *TP53* mutational status during hydroxyurea treatment

✓ Determine the optimal age for predicting the appearance of *TP53* multihit events

CONTACT INFORMATION

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