TET2 Mutation in cellular reprogramming and hematopoietic differentiation

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INTRODUCTION

Primary myelofibrosis (PMF) is characterized by an increased myeloproliferation and bone marrow fibrosis. In PMF, driver somatic mutations occur in *JAK2, MPL* or *CALR* genes and mutations in epigenetic regulators as *TET2* and *ASXL1* that could leave to loss-of-function were frequently identified in PMF patients. In this context, induced pluripotent stem (iPS) cells could be used to recapitulate in vitro the disease phenotype, to study clonal heterogeneity and drug efficacy. The main goal of this work was to assess the impact of somatic mutations in *CALR* and *TET2* in both cellular reprogramming and hematopoietic differentiation using the iPS cells.

MATERIAL AND METHODS

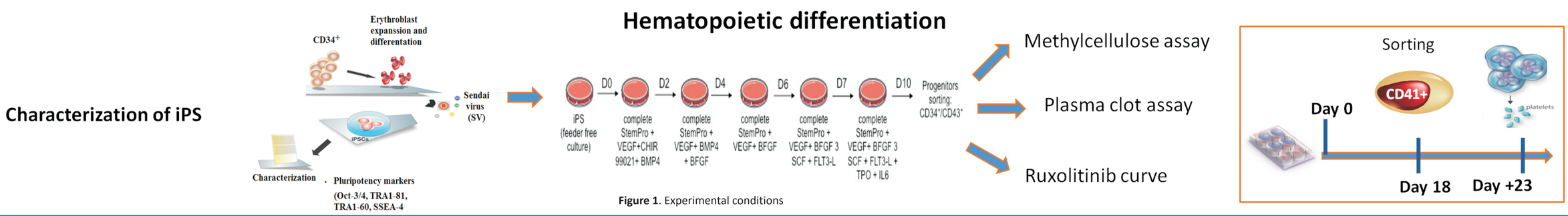


Figure 1. Experimental conditions

RESULTS

Molecular Characterization

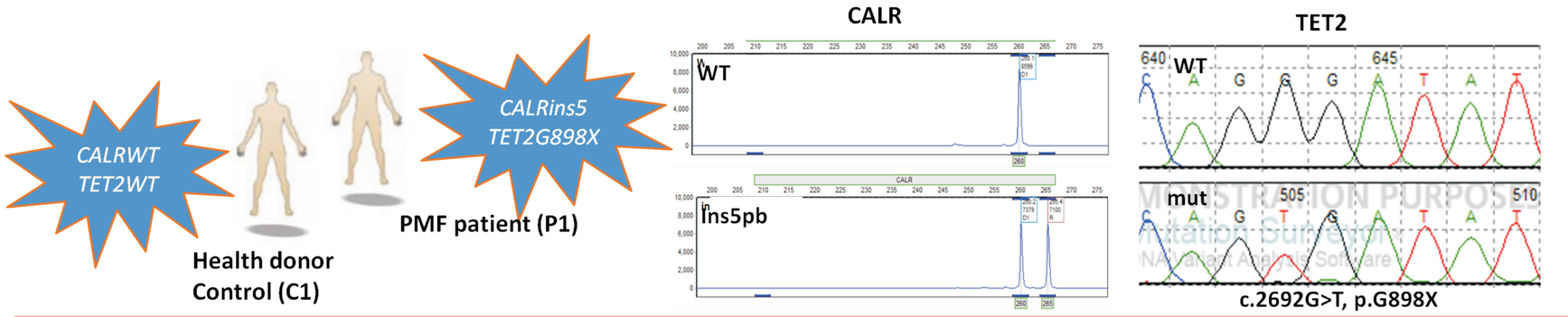


Tabela 2. Number of iPS observed at day 21 of reprogramming.

	Genotype (iPS)	N° iPS
Control	iPS (10)	148
Patient P1	$CALR^{ins5}/TET2^{wt}$ (20) $CALR^{ins5}/TET2^{G898X}$ (12)	32

The pluripotency status of iPS

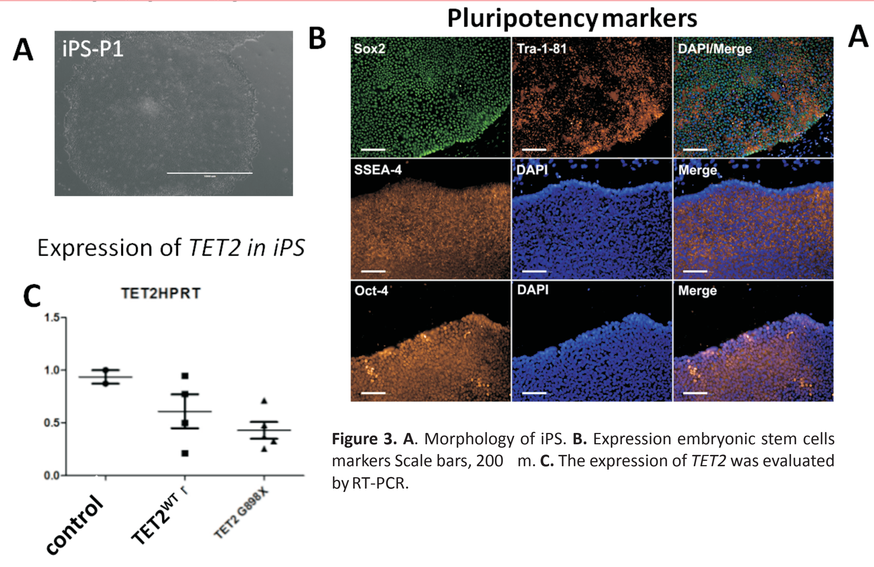


Figure 3. A. Morphology of iPS. **B.** Expression embryonic stem cells markers Scale bars, 200 m. **C.** The expression of *TET2* was evaluated by RT-PCR.

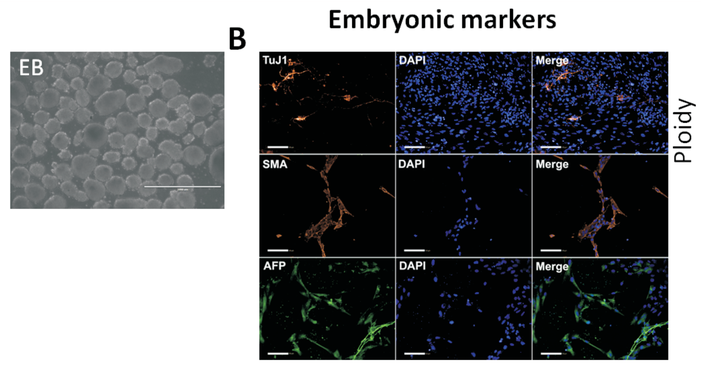


Figura 4. A. Embryonic body (EB) formation. **B.** The capacity to form germ layers was evaluated by embryonic body (EB) formation and layer specific markers detection **C.** Analysis of Sendai virus (SV) expression. Transduced cell pool at passage 3 was used as positive control. C- , non-template control.

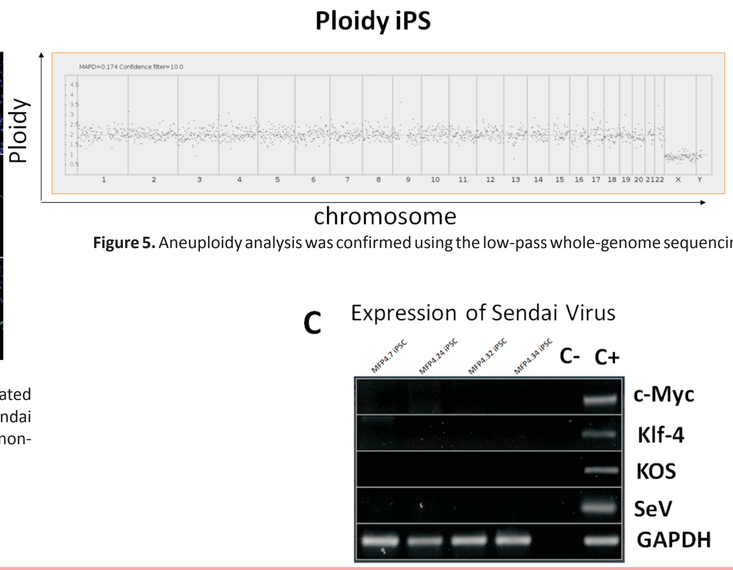


Figure 5. Aneuploidy analysis was confirmed using the low-pass whole-genome sequencing.

Hematopoietic differentiation

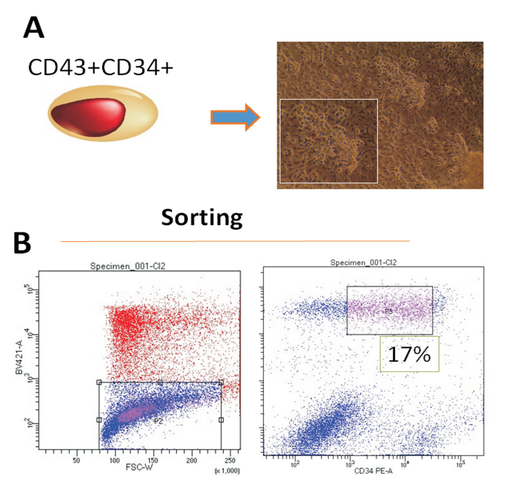


Figure 6. A. Image of hematopietic differentiation at D+9. **B.** CD43+CD34+ progenitors were sorted at D+10.

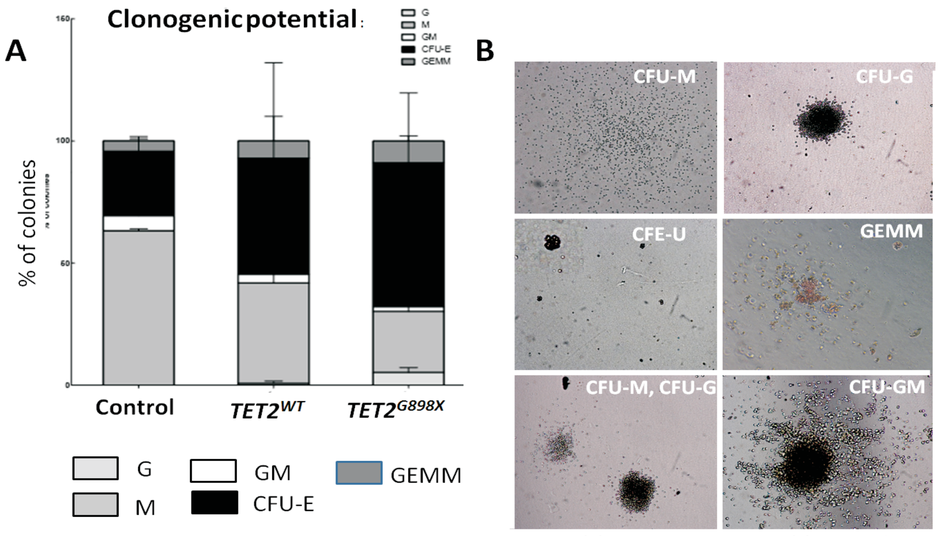
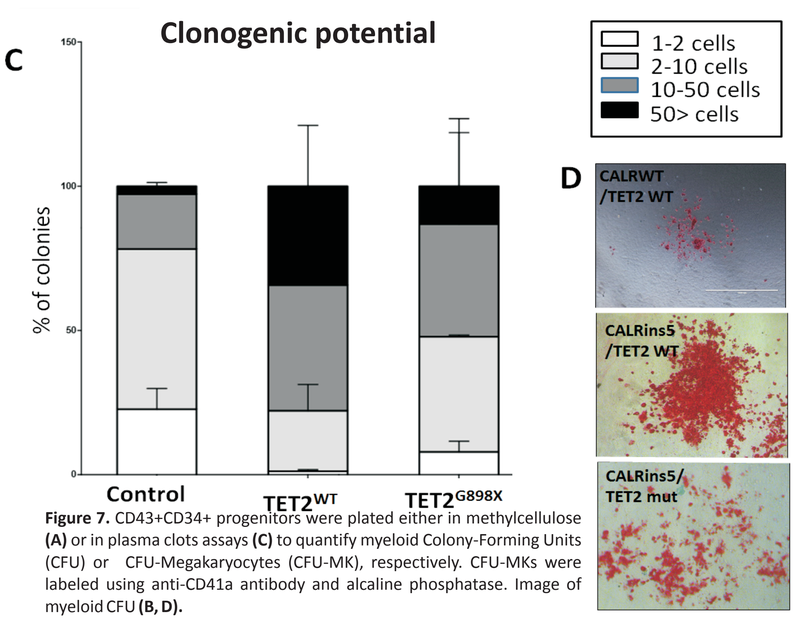


Figure 7. CD43+CD34+ progenitors were plated either in methylcellulose **(A)** or in plasma clots assays **(C)** to quantify myeloid Colony-Forming Units (CFU) or CFU-Megakaryocytes (CFU-MK), respectively. CFU-MKs were labeled using anti-CD41a antibody and alcaline phosphatase. Image of myeloid CFU **(B, D).**



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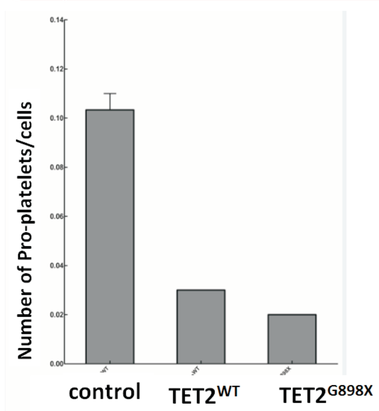
Pro-platelets formation



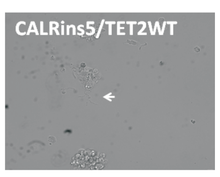
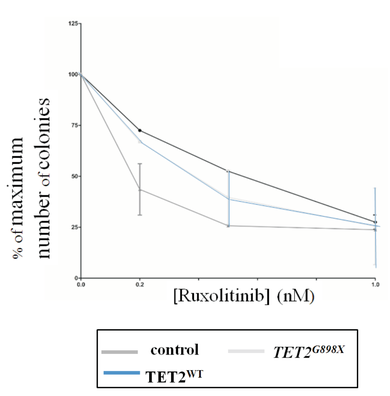


Figure 8. The number of pro-platelets forming cells was counted by microscopy.

Ruxolitinibe



CONCLUSION

Our results suggests that the mutation in $TET2^{G898X}$ did not seem to impair cellular reprogramming, since iPS cells harboring these mutation display all the features of bona fide iPS and that mutation in $CALR^{ins5}$ sem to impact hematopoietic differentiation of iPS cells. Finally, we observed by plasma clot assays that clone iPS CALRins5/TET2mut resulted more sensitive to Ruxolitinib vs C1, suggesting a dependence on JAK/STAT signaling. Theses results could help to understand the role of the described mutations in the pathophysiology of PMF.

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