The telomerase inhibitor imetelstat differentially targets JAK2V617F versus CALR mutant myeloproliferative neoplasm cells and inhibits JAK-STAT signaling – P1008

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INTRODUCTION

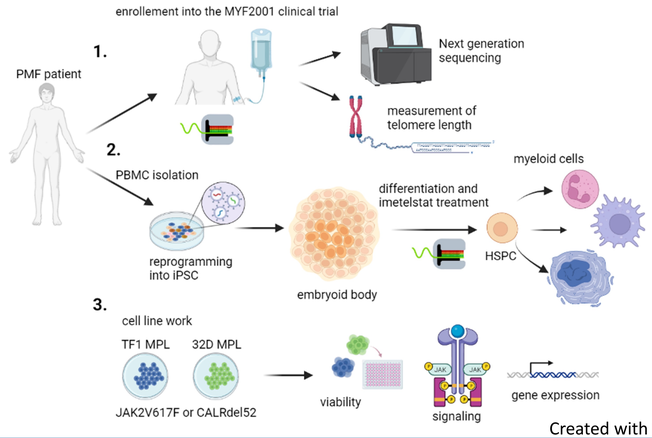
Imetelstat, a telomerase inhibitor, has shown clinical activity in patients with Myeloproliferative Neoplasms (MPN), including primary myelofibrosis (PMF) and essential thrombocythemia (ET).[1, 2, 3] Imetelstat is an oligonucleotide with a nucleotide sequence that is complementary to and therefore specifically binds to the template region of the RNA component of human telomerase with high affinity and acts as a potent, competitive inhibitor of telomerase enzymatic activity. Although inhibition of telomerase by imetelstat leads to telomere length (TL) shortening, its mechanisms to induce responses in MPN need further elucidation.

OBJECTIVES

- To analyze the MPN mutation profile during a two-year course of imetelstat treatment as a means to assess clonal evolution and disease progression in a patient with MF.
- To study mechanistic differences of imetelstat response in MPN cells harboring JAK2V617F versus CALRdel52 mutations *in vitro*

METHODS

1) A high-risk PMF patient with multiple disease-related mutations who had been heavily pre-treated (e.g., ruxolitinib and hydroxyurea combination) when enrolled into imetelstat clinical trial MYF2001
   - ➔ TL was measured via flow-FISH during 30 months of imetelstat treatment
   - ➔ Mutational profile was assessed during 7.5 years pre- and post-imetelstat treatment
2) Reprogramming of patient-derived peripheral blood mononuclear cells (PBMCs), generation of disease-specific induced pluripotent cells (iPSCs)
3) Imetelstat response was analyzed in human TF-1^{MPL} and murine 32D^{MPL} cells stably expressing JAK2V617F or CALRdel52



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RESULTS

Clonal evolution of PMF during over 7 years of treatment.

Table 1: Variant allele frequency of MPN-related mutations of clinical significance in MPN patient and corresponding iPSC

	*JAK2*V617F	*ASXL1*T880fs	*ASXL1* R965*	*KRAST*58I	*TET2*E1470fs	*U2AF1*Q157R
Patient (year 1, before RUX treatment)	50%	32%	8.8%	Neg (<1%)	42%	42%
Patient (year 3, before imetelstat treatment)	57%	36%	9.1%	2.53%	46%	45%
Patient (year 5, at time of iPSC generation)**	48%	45%	Neg (<1%)	52%	44%	45%
iPSC (generated from year 5 cells)**	51%	40%	Neg (<1%)	50%	46%	49%
Patient (year 7)***	53%	48%	Neg (<1%)	54%	48%	49%
Patient (year 7.5)	55%	48%	Neg (<1%)	62%	47%	47%

Abbreviations: * - Substitution; fs – frameshift; iPSC – induced pluripotent stem cells; Neg – negative; RUX – ruxolitinib; ** on imetelstat; *** off imetelstat

Efficient reduction of telomere length by imetelstat *in vivo* and of patient-specific iPSC-derived HSPC *in vitro*.

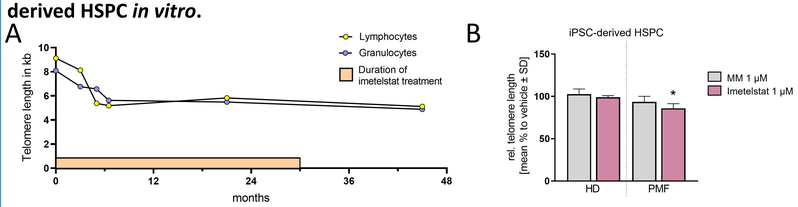

Figure 1. (A) Telomere length of a PMF patient treated with imetelstat (9.4 mg/kg) for 30 months in the MYF2001 clinical trial was measured via flow-FISH. **(B)** Telomere length of iPSC-derived CD34+ HD or PMF cells treated with 1 µM MM, 1 µM imetelstat or vehicle for 14 days. Telomere length is shown relative to vehicle control. *p<0.05, n=3. HD: healthy donor; HSPC: hematopoietic stem and progenitor cells; iPSC: induced pluripotent stem cell; MM: mismatch control; SD: standard deviation

Imetelstat reduces clonogenic capacity of primary CD34+ cells, while iPSC-derived CD34+ cells and iPSC-derived megakaryocytes remained unaltered.

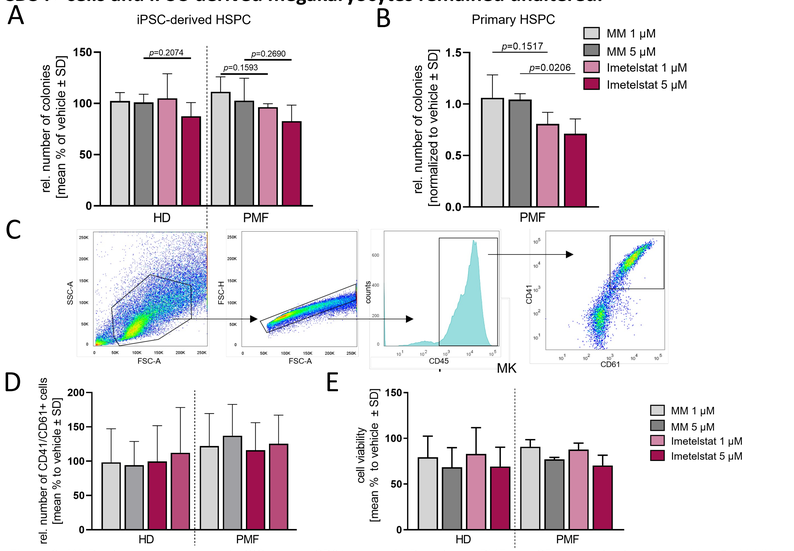

Figure 2. (A) Relative colony number of iPSC-derived CD34+ cells of HD control or PMF in a CFU assay treated with 1 µM or 5 µM MM or imetelstat. n=3. **(B)** Relative colony number in a CFU assay of primary CD34+ cells treated with 1 µM or 5 µM of MM or imetelstat. n=3. **(C)** Representative gating strategy to evaluate number of CD41/CD61+ megakaryocytic cells after 14 days of differentiation. **(D)** Number of iPSC-derived CD41+/CD61+ megakaryocytic cells after 6 days of treatment with vehicle control, 1 or 5 µm MM or imetelstat during differentiation. Number of megakaryocytic cells was compared to vehicle control. n=3. **(E)** Drug response on cell viability of control HD or PMF iPSC-derived CD61+ megakaryocytes treated with 1 or 5 µM MM or imetelstat for 72 h. n=3.

RESULTS

Imetelstat shows stronger effects in *CALR*del52- than *JAK2*V617F-mutated cells and acts via JAK-STAT signaling.

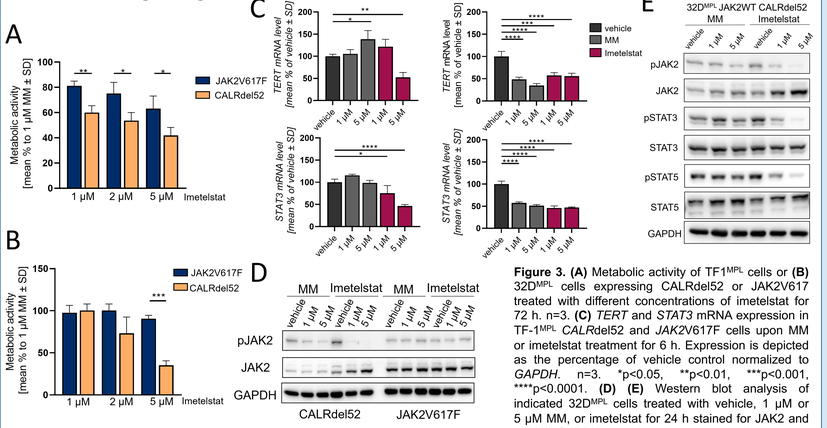

Figure 3. (A) Metabolic activity of TF1^{MPL} cells or **(B)** 32D^{MPL} cells expressing CALRdel52 or JAK2V617 treated with different concentrations of imetelstat for 72 h. n=3. **(C)** *TERT* and *STAT3* mRNA expression in TF-1^{MPL} *CALR*del52 and *JAK2*V617F cells upon MM or imetelstat treatment for 6 h. Expression is depicted as the percentage of vehicle control normalized to *GAPDH*. n=3. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **(D)** **(E)** Western blot analysis of indicated 32D^{MPL} cells treated with vehicle, 1 µM or 5 µM MM, or imetelstat for 24 h stained for JAK2 and pJAK2. GAPDH served as a loading control.

CONCLUSIONS

Our data demonstrate that imetelstat reduces TL and targets JAK/STAT signaling, particularly in CALR-mutated cells. Imetelstat reduced clonogenic growth of the patient's primary but not iPSC-derived CD34+ cells, suggesting that the iPSC reprogramming-associated TL increase (from 5.8 kb and 5.49 kb in lymphocytes and granulocytes, respectively, to 13.9 kb ± 1.85 in iPSC-derived HSPC) lowers the cells´ sensitivity to telomerase inhibition. In addition, we observed the expansion of a KRAST58I mutated clone during imetelstat treatment, and thus the role of KRAS mutations in the cellular response to imetelstat needs further investigation. Importantly, although the exact patient subpopulation who will benefit most from imetelstat is still to be defined, our data propose that CALR-mutated clones are highly vulnerable to imetelstat.

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