Imetelstat (GRN163L), a Telomerase Inhibitor Selectively Affects Megakaryopoiesis in Myeloproliferative Neoplasms (MPN)

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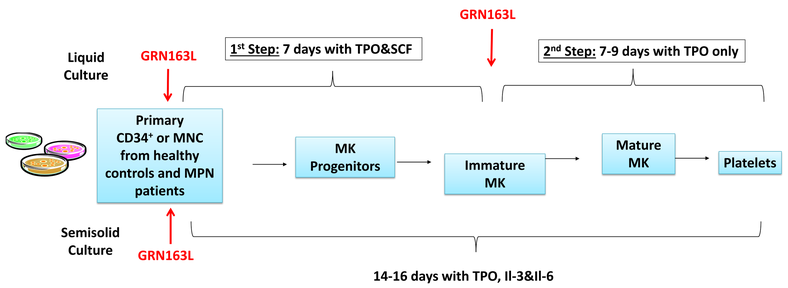
ABSTRACT

Imetelstat is a telomerase inhibitor which has been shown to have therapeutic activity in patients with myelofibrosis (MF) (*Tefferi A. et al., 2013 ASH Annual Meeting, Abstract 662*) and essential thrombocythemia (ET) (*Baerlocher G. et al., 2012 ASH Annual Meeting, Abstract 179*). In clinical trials involving patients with a variety of other malignancies, the primary dose-limiting toxicity of GRN163L has been thrombocytopenia. We utilized GRN163L in order to explore the possible role of telomerase on human megakaryocytes (MK) and the mechanisms underlying the drug's inhibitory effects on platelet production. MK were generated from normal primary CD34$^+$ cells using an ex vivo culture system previously described by our laboratory *(Iancu-Rubin C. et al, Blood 2011, 117:17; 4580-4589)*. We first showed that both telomerase activity and the expression of its catalytic unit hTERT were elevated in proliferating normal CD34$^+$ cells, declined transiently during the initial stages of MK differentiation but were then partially restored during the final stages of terminal maturation. Treatment of normal CD34$^+$ cells with GRN163L did not affect the numbers of CFU-MK assayed. Furthermore, exposure to GRN163L during the first 7 days in a liquid culture system did not interfere with the ability of CD34$^+$ cells to commit to MK. When the same cultures were allowed to mature for 7 additional days, the proportion of CD34$^+$/CD41$^+$ MK precursors in drug-treated cultures was twice that observed in control and the drug-treated cultures contained 70% fewer mature CD41$^+$/CD42b$^+$ MK than control cultures. The inhibitory effects of GRN163L on MK maturation were supported by observations showing that the cultures treated with the drug contained 60% fewer polyploid MK than control cultures. These results suggest that that GRN163L-mediated inhibition of telomerase does not affect normal MK progenitors but blocks the maturation of MK precursor cells.

Previous studies have shown that GRN163L inhibits neoplastic CFU-MK colony formation by CD34$^+$ cells from patients with ET (*Brunold C. et al., 2011 ASH Annual Meeting, Abstract 3843*). We, therefore, examined the effects of GRN163L on malignant MK from patients with MF and ET. Unlike normal CD34$^+$ cells, treatment of MPN PB-MNCs with GRN163L decreased the numbers of assayable CFU-MK from 6 out of 11 patients. CFU-MK colony formation by PB-MNCs from these 6 patients was reduced by 33% (ranging from 13% to 50% reduction in CFU-MK formation) as compared to PB-MNCs treated with an inactive form of the drug (p value= 0.00473). Of note, in two out of 5 patients in which the total number of CFU-MK was not affected by GRN163L treatment, the drug decreased the size of the CFU-MK colonies formed. The ability of MPN PB-MNCs to differentiate into MK was next assessed. Although the total number of cells in PB-MNC liquid cultures exposed to GRN163L was decreased as compared to those treated with the inactive drug (n=6; p value=0.03204), the proportion of CD34$^+$/CD41$^+$ MK precursors generated was not significantly affected. We then evaluated the effects of GRN163L on the genotype of MPN MK generated in the presence and absence of GRN163L by assessing the JAK2V617F allele burden. Treatment with GRN163L but not the inactive form of the drug reduced the JAK2V617F allele burden in MK derived from two patients: in one patient, JAK2V617F allele burden in MK generated in the presence of the inactive drug was 91.86% while MK generated in the presence of GRN163L was 19.75%; MK generated from a second patient had a JAK2V617F allele burden of 10.84% in the presence of the inactive drug which was reduced to 2.14% in the presence of GRN163L.

We conclude that GRN163L-mediated inhibition of telomerase affects normal megakaryopoiesis by blocking the terminal maturation of CD34$^+$/CD41$^+$ MK precursors, providing a possible explanation for GRN163L's propensity to induce thrombocytopenia in patients with normal marrow. By contrast, GRN163L treatment inhibited the ability of MPN CD34$^+$ cells but not normal CD34$^+$ cells to form CFU-MK colonies and drug treatment reduced the numbers of malignant MK generated. We propose that the amelioration of fibrosis observed in a clinical trial of MF patients treated with GRN163L might be due to, at least in part, two potential modes of action: **1)** inhibiting malignant MK progenitors cells and **2)** preventing terminal MK maturation thus depleting the pool of mature MKs which are the major source of fibrogenic cytokines in MF.

EXPERIMENTAL DESIGN



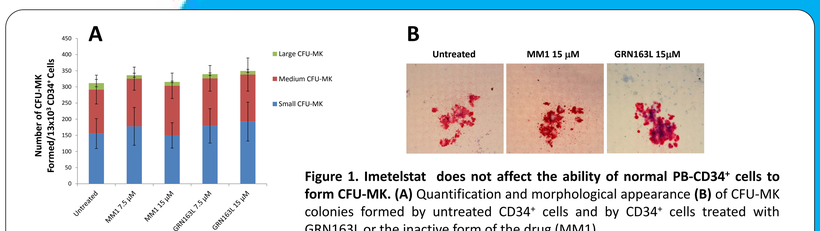


Figure 1. Imetelstat does not affect the ability of normal PB-CD34$^+$ cells to form CFU-MK. (A) Quantification and morphological appearance **(B)** of CFU-MK colonies formed by untreated CD34$^+$ cells and by CD34$^+$ cells treated with GRN163L or the inactive form of the drug (MM1).

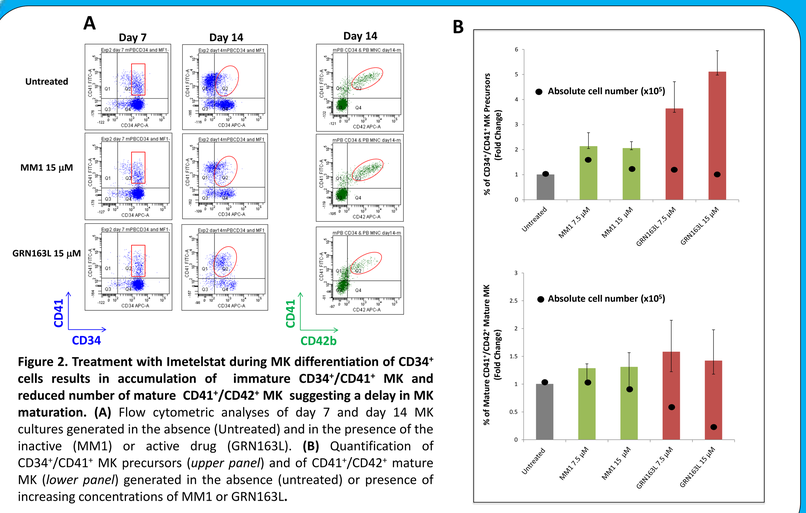


Figure 2. Treatment with Imetelstat during MK differentiation of CD34$^+$ cells results in accumulation of immature CD34$^+$/CD41$^+$ MK and reduced number of mature CD41$^+$/CD42$^+$ MK suggesting a delay in MK maturation. (A) Flow cytometric analyses of day 7 and day 14 MK cultures generated in the absence (Untreated) and in the presence of the inactive (MM1) or active drug (GRN163L). **(B)** Quantification of CD34$^+$/CD41$^+$ MK precursors (*upper panel*) and of CD41$^+$/CD42$^+$ mature MK (*lower panel*) generated in the absence (untreated) or presence of increasing concentrations of MM1 or GRN163L.

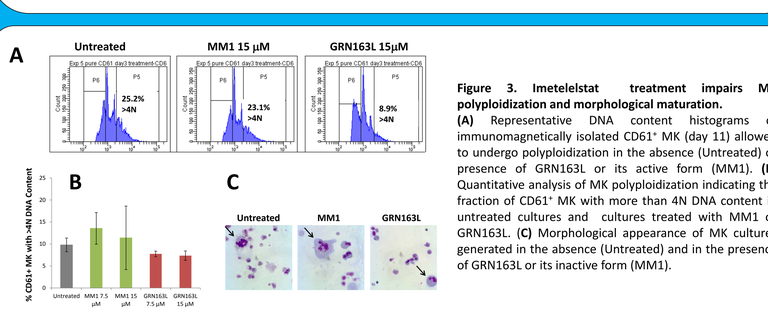


Figure 3. Imetelelstat treatment impairs MK polyploidization and morphological maturation.
(A) Representative DNA content histograms of immunomagnetically isolated CD61$^+$ MK (day 11) allowed to undergo polyploidization in the absence (Untreated) or presence of GRN163L or its active form (MM1). **(B)** Quantitative analysis of MK polyploidization indicating the fraction of CD61$^+$ MK with more than 4N DNA content in untreated cultures and cultures treated with MM1 or GRN163L. **(C)** Morphological appearance of MK cultures generated in the absence (Untreated) and in the presence of GRN163L or its inactive form (MM1).

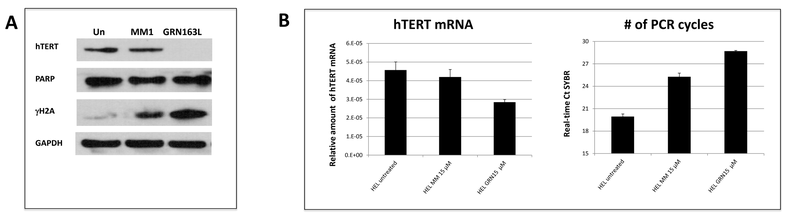


Figure 4. Imetelstat inhibits telomerase expression (hTERT) and activity (TA) and induces DNA damage in HEL JAK2V617F cells.
(A) Western blot analyses of protein lysate from HEL JAK2V617F cells untreated (Un) or treated with GRN163L or with its inactive form (MM1). **(B)** hTERT mRNA expression and telomerase activity (TA) in untreated and treated HEL JAK2V617F cells. TA was evaluated by a PCR-based assay in which the relative enzymatic activity is determined based on the enzyme's ability to synthetize telomeric repeats which were quantified by PCR; the higher the number of PCR cycles (C_t SYBR) required for telomeric sequences amplification, the lower the TA.

Efficiency of CFU-MK formation			
	MM1	GRN163L	Outcome
Pt2	99.3	54.8	45% reduction
Pt3	92	94	No response
Pt141	78.5	91	No response for total CFU-MK number Reduced CFU-MK size
Pt59	54.8	66	No response for total CFU-MK number Reduced CFU-MK size
Pt49	99	49.6	50% reduction
Pt138	145	97.9	32.5% reduction
Pt5	9.5	20.5	No Response
Pt94	129.7	141.4	No response
Pt7	96.6	93	13% reduction
PT8	97.5	84.1	14% reduction
Pt9	119.6	66.6	44% reduction

Table 1. Imetelstat treatment inhibits CFU-MK formation by MPN MNC. PB-MNC from MPN patients were plated in collagen-based MegaCult media (Stem Cell Technologies) in the absence or presence of drugs. After 14 days, CFU-MK formed were labeled with anti-GPIIIa/IIb antibodies and enumerated. CFU-MK formation efficiency was determined by normalizing the numbers of CFU-MK found in treated cultures to those found in untreated conditions which were considered 100% CFU-MK formation efficiency.

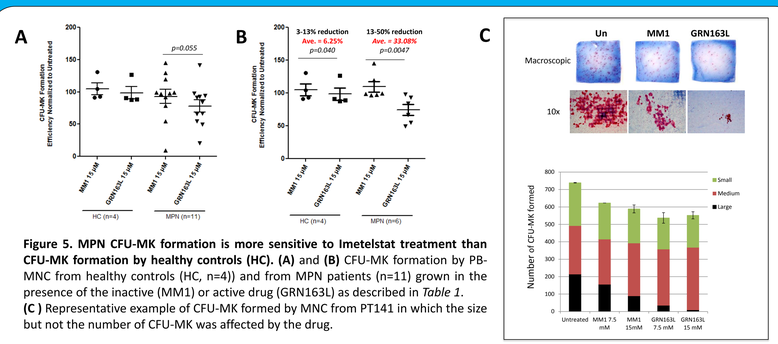


Figure 5. MPN CFU-MK formation is more sensitive to Imetelstat treatment than CFU-MK formation by healthy controls (HC). (A) and **(B)** CFU-MK formation by PB-MNC from healthy controls (HC, n=4)) and from MPN patients (n=11) grown in the presence of the inactive (MM1) or active drug (GRN163L) as described in *Table 1*.
(C) Representative example of CFU-MK formed by MNC from PT141 in which the size but not the number of CFU-MK was affected by the drug.

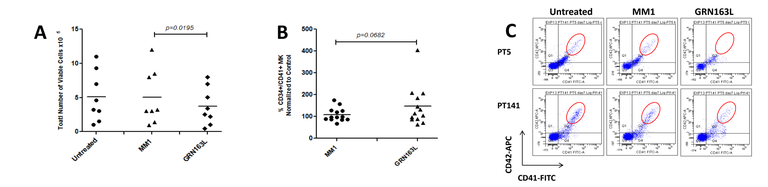


Figure 6. Imetelstat impairs MPN megakaryopoiesis by reducing the number of MK generated and by preventing their terminal maturation. (A) and **(B)** PB-MNCs from MPN patients (n=8) were plated in liquid culture in the absence (untreated) and in the presence of inactive (MM1) or active drug (GRN163L). After 7 days, viable cells were enumerated (A) and the percentage of CD34$^+$/CD41$^+$ MK precursors (B) was determined by flow cytometry. (C) Representative examples of dot plot analyses of mature MK (CD41$^+$/CD42$^+$) generated after 14 days in culture by PB-MNC from two MPN patients.

	JAK2V617F Allele Burden (%)	
	Untreated	GRN163L-treated
Pt6	34.37	19.75
Pt9	7.14	2.14
Pt141	45.17	42.14

Table 2. Imetelstat treatment reduces JAK2V617F allele burden in MK derived from MPN MNC. Mutated JAK2 allele burden was determined on DNA extracted from untreated or GRN163L-treated MK cultures generated by PB-MNC from three JAK2V617F-positive MPN patients.

SUMMARY AND CONCLUSIONS

- Imetelstat-mediated inhibition of telomerase affects normal megakaryopoiesis by blocking the terminal maturation of CD34$^+$/CD41$^+$ MK precursors, providing a possible explanation for Imetelstat's propensity to induce thrombocytopenia in patients with normal marrow.
- Imetelstat treatment inhibited the ability of MPN CD34$^+$ cells but not normal CD34$^+$ cells to form CFU-MK colonies and drug treatment reduced the numbers of malignant MK generated.

We propose that the amelioration of fibrosis observed in a clinical trial of MF patients treated with Imetelstat might be due to, at least in part, two potential modes of action:
1) inhibiting malignant MK progenitors cells
2) preventing terminal MK maturation thus depleting the pool of mature MK which are the major source of fibrogenic cytokines in MF

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