Telomerase Inhibition with Imetelstat Eradicates β-catenin Activated BC CML Stem Cells

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INTRODUCTION

Leukemia stem cells (LSCs) in chronic myeloid leukemia (CML) are generated from progenitors that have aberrantly activated self-renewal pathways thereby resulting in tyrosine kinase inhibitor (TKI) resistance. The telomerase complex, consisting of a reverse transcriptase subunit (TERT), an RNA template subunit (TERC), and a protective shelterin scaffold, transcriptionally modulates the Wnt/β-catenin self-renewal pathway. Many malignancies, including BCR-ABL TKI resistant blast crisis (BC) CML, exhibit robust telomerase activity, present at very low or undetectable level in normal cells. Furthermore, telomerase plays a pivotal role in cancer cell growth, and may serve as an ideal target for anticancer therapeutics, which prompted the development of imetelstat, a competitive inhibitor of telomerase enzymatic activity. Imetelstat is a lipidated 13-mer oligo-nucleotide that binds with high affinity to the TERC subunit.

Recent clinical trials showed early signs of efficacy in myelofibrosis [1] and essential thrombocythemia [2]. However, the role of imetelstat in selectively inhibiting LSC self-renewal in CML had not been elucidated.

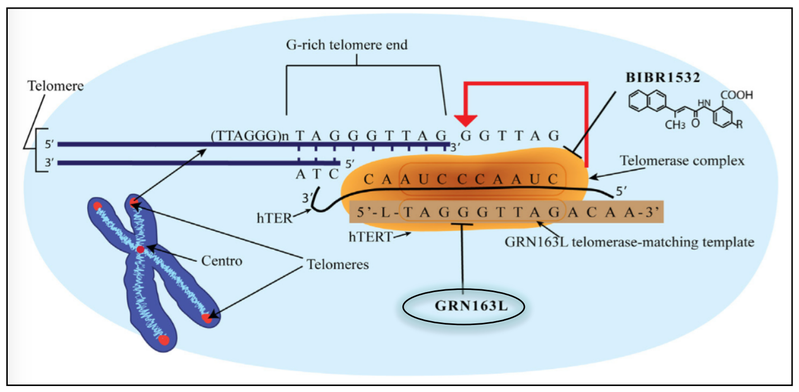

Fig. 1 Imetelstat (GRN163L) targets the RNA template of telomerase, binding with high specificity and affinity, leading to a complete inhibition of the enzyme [3].

OBJECTIVES

In order to investigate the capacity of imetelstat to selectively inhibit LSC self-renewal and to determine the mechanism of action, stromal co-cultures and humanized LSC primagraft studies were performed.

METHODS & RESULTS

Human LSC-supportive SL/M2 stromal co-cultures were set up for assaying in vitro self-renewal. Humanized mouse models of BC CML and normal stem cells were established and treated with vehicle, mis-match control and imetelstat at 30 mg/kg, 3 times a week for 4 weeks; qRT-PCR was used for measuring β-catenin transcript levels in the samples treated with mis-match control (m/m) and imetelstat (IMS). FACS analysis was applied for measuring the levels of tumor engraftment and activated β-catenin protein in engrafted mouse bone marrow (BM), spleen (SP) and human myeloid progenitors.

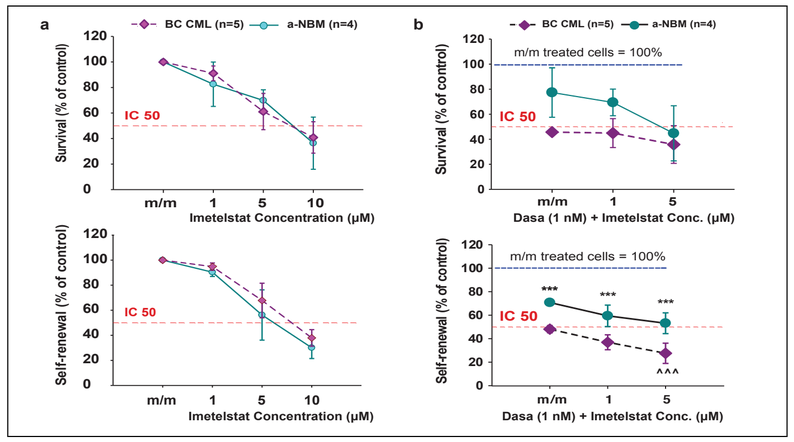

Fig. 2 Imetelstat alone induced a dose-dependent inhibition of self-renewal in aged normal bone marrow (a-NBM) and BC CML (a). Combined treatment with a low dose of dasatinib (1 nM) and imetelstat doses of 1 or 5 μM resulted in a significant difference (***, ^^^ $p<0.001$, Anova) in self-renewal of BC CML cells (b).

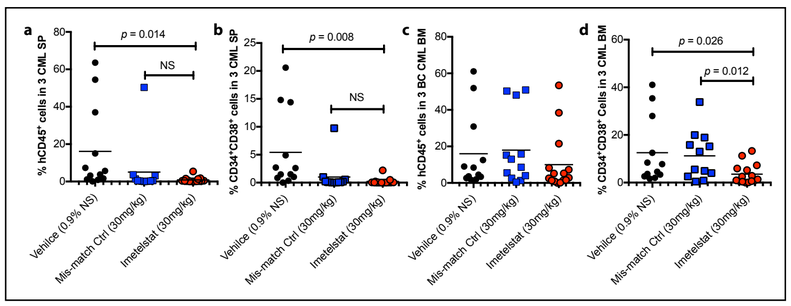

Fig. 3 Human CD45$^+$ and CD34$^+$CD38$^+$ cells were significantly inhibited in both BM and SP after imetelstat treatment. Human CD45$^+$ cells were significantly inhibited in SP (a), CD34$^+$CD38$^+$ cells were significantly inhibited in both SP (b) and BM (d) after imetelstat treatment in comparison to vehicle control. Student's *t*-tests were applied to the groups.

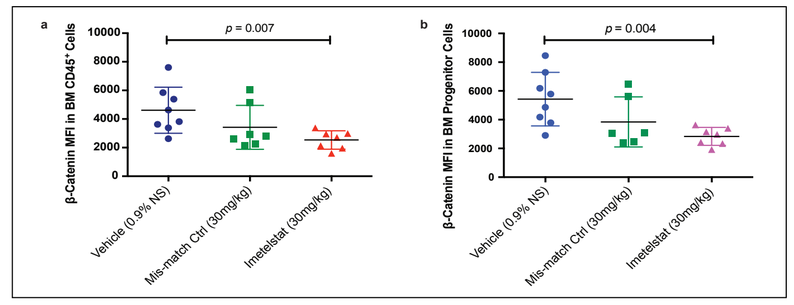

Fig. 4 Inhibition of β-catenin in humanized BC CML LSC mouse BM. β-catenin was significantly inhibited in human CD45$^+$ cells (a) and progenitors (b) in BC CML PDX mouse BM after imetelstat treatment in comparison to vehicle control treatment.

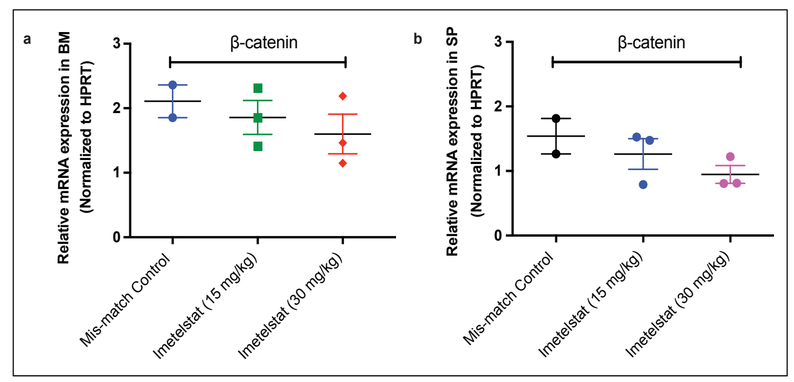

Fig. 5 β-catenin mRNA levels were inhibited in both BM (a) and spleen (b) after imetelstat treatment in comparison to the controls, determined by RT-qPCR. hCD45$^+$ cells were isolated from the PDX mouse BM & spleen.

CONCLUSIONS

Niche responsive interactions between the telomerase complex and the Wnt/β-catenin self-renewal pathway sensitize β-catenin activated LSC to imetelstat in both in vitro and in vivo humanized pre-clinical BC CML models, and spares to normal stem cells (not shown) providing a strong rationale for LSC eradication trials involving imetelstat.

REFERENCES

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3. Ruden M, et al. Cancer Treatment Rev. 2013; 39: 444-456

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