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 lipiddated 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.

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, mismatch 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 mixed-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.

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