



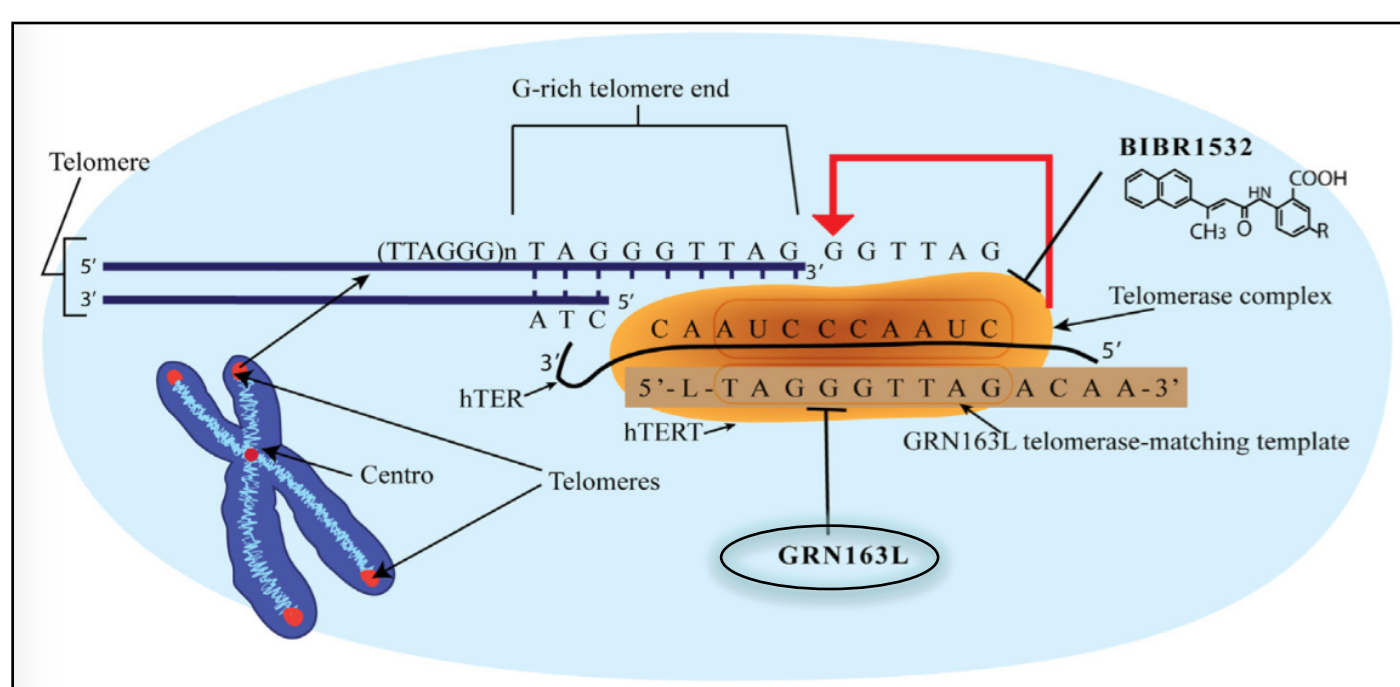
# Telomerase Inhibition with Imetelstat Eradicates $\beta$ -catenin Activated BC CML Stem Cells

Wenxue Ma<sup>1</sup>, Cayla Mason<sup>1</sup>, Ping Chen<sup>1</sup>, Nathaniel Delos Santos<sup>1</sup>, Qingfei Jiang<sup>1</sup>, Elisa Lazzari<sup>1</sup>, Fei Huang<sup>2</sup>, Larisa Balaian<sup>1</sup>, and Catriona Jamieson<sup>1</sup>  
<sup>1</sup> Division of Regenerative Medicine and Moores Cancer Center, University of California San Diego, La Jolla, CA; <sup>2</sup> Janssen Research & Development, LLC

## 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/ $\beta$ -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.



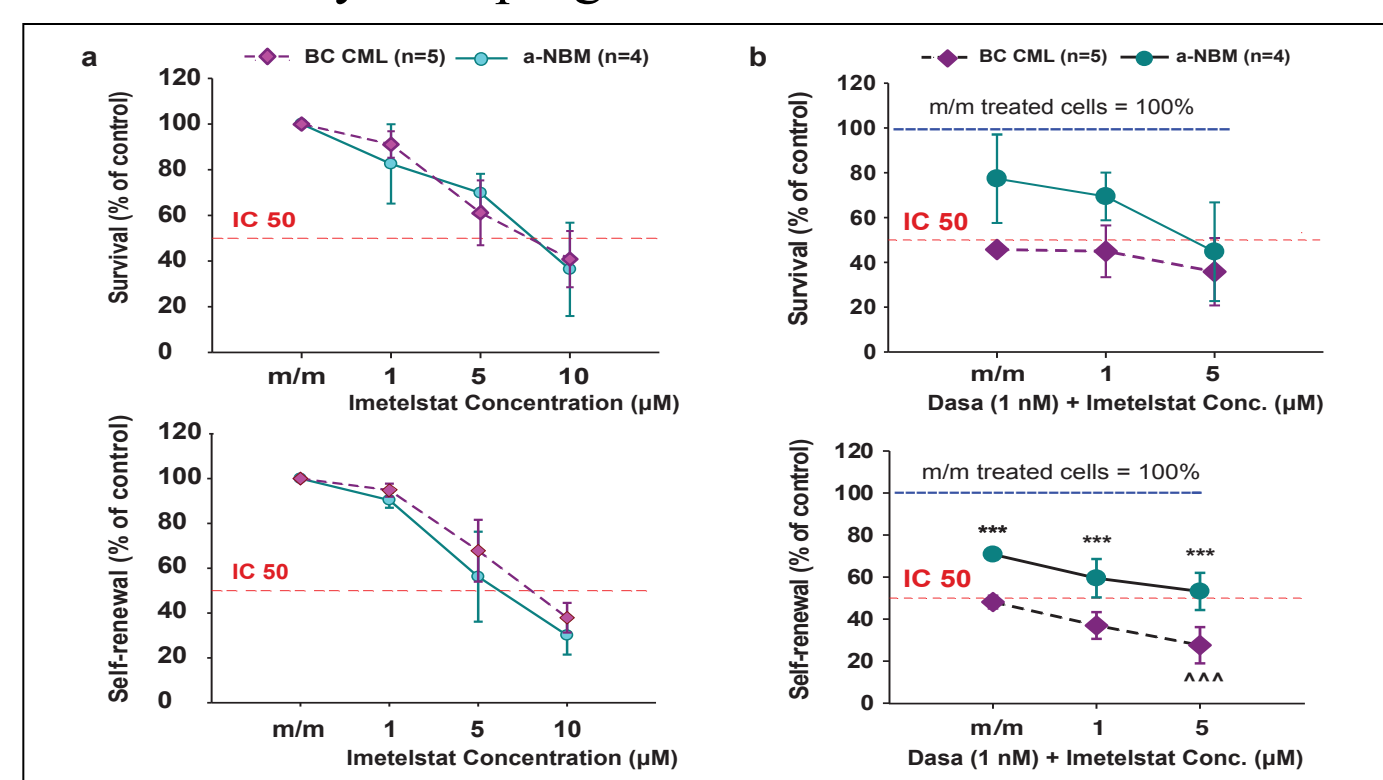
**Fig. 1** Imetelstat (GRN163L) targets the RNA template of telomerase, binding with high specificity and affinity, leading to a complete inhibition of the enzyme [3].

## OBJECTIVES

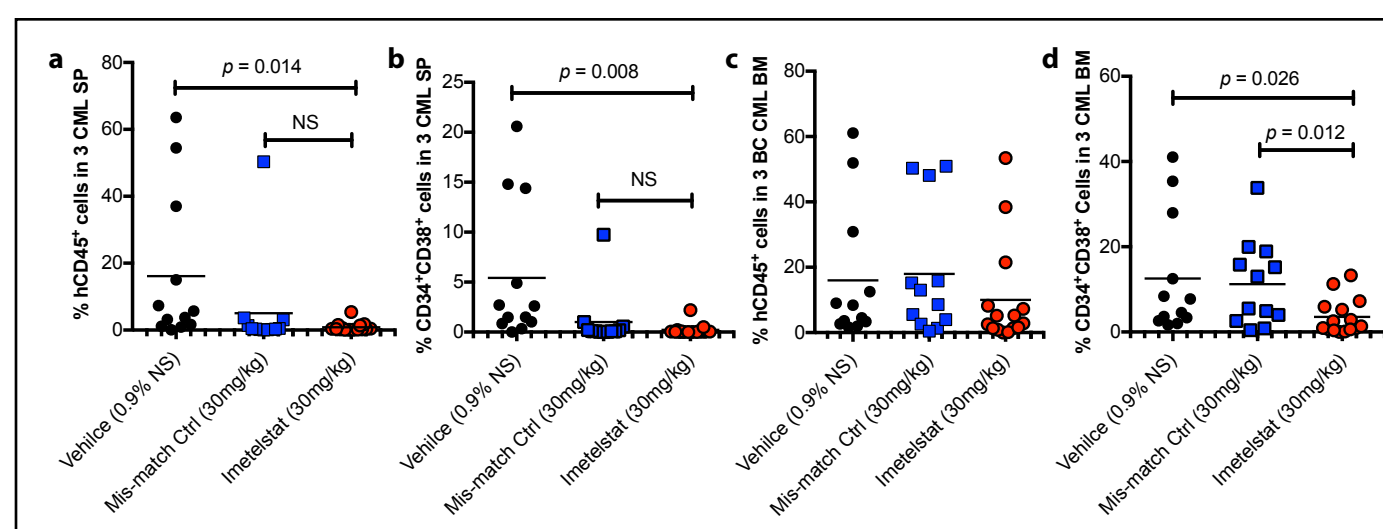
In order to investigate the capacity of imetelstat to selectively inhibit LSC self-renewal and to determine the mechanism of action, stromal co-cultures and humanized LSC primagraft studies were performed.

## 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  $\beta$ -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  $\beta$ -catenin protein in engrafted mouse bone marrow (BM), spleen (SP) and human myeloid progenitors.

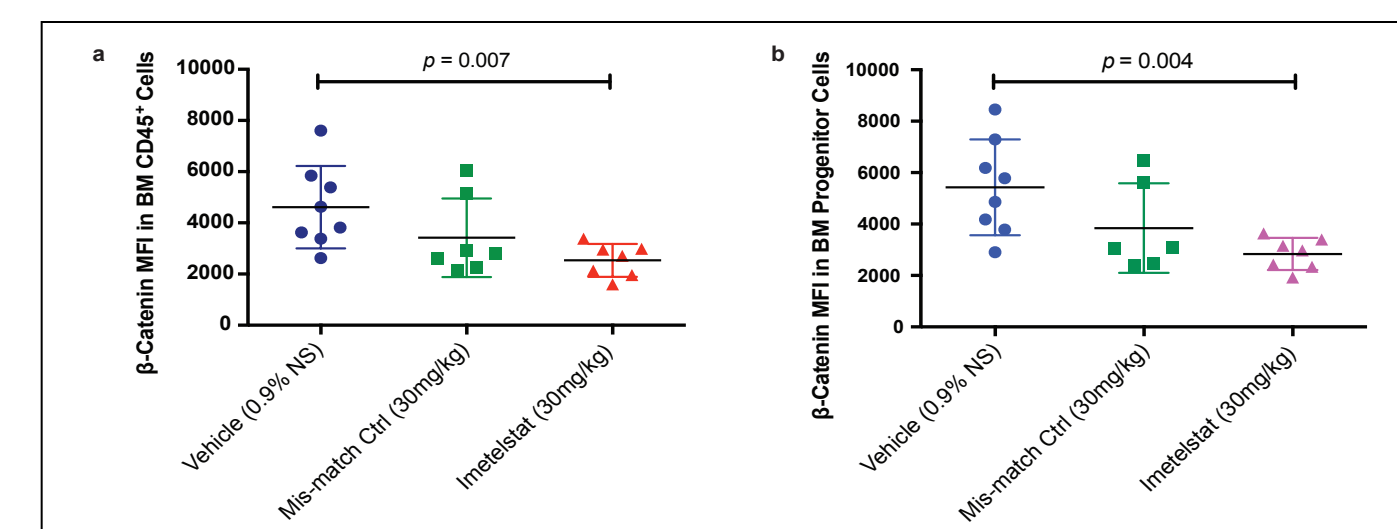


**Fig. 2** Imetelstat alone induced a dose-dependent inhibition of self-renewal in aged normal bone marrow (a-NBM) and BC CML (a). Combined treatment with a low dose of dasatinib (1 nM) and imetelstat doses of 1 or 5  $\mu$ M resulted in a significant difference (\*\*\*, ^^^  $p < 0.001$ , Anova) in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

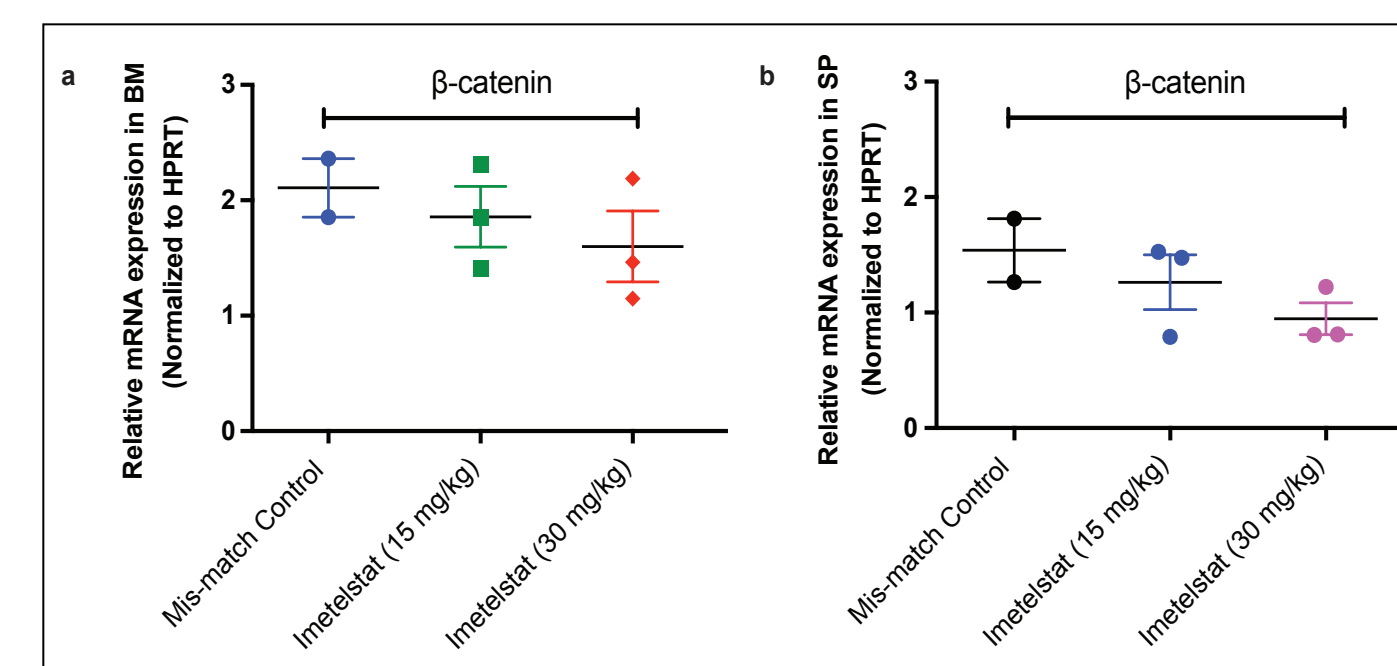


**Fig. 3** Human CD45<sup>+</sup> and CD34<sup>+</sup>CD38<sup>+</sup> cells were significantly inhibited in both BM and SP after imetelstat treatment. Human CD45<sup>+</sup> cells were significantly inhibited in SP (a), CD34<sup>+</sup>CD38<sup>+</sup> cells were significantly inhibited in both SP (b)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

## RESULTS



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



**Fig. 5**  $\beta$ -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<sup>+</sup> cells were isolated from the PDX mouse BM & spleen.

## CONCLUSIONS

Niche responsive interactions between the telomerase complex and the Wnt/ $\beta$ -catenin self-renewal pathway sensitize  $\beta$ -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

1. Tefferi A, et al. *N Engl J Med.* 2015; 373: 908-919
2. Baerlocher G, et al. *N Engl J Med.* 2015; 373: 920-928
3. Ruden M, et al. *Cancer Treatment Rev.* 2013; 39: 444-456