Telomerase Inhibition Impairs Self-renewal of β-catenin Activated Myeloproliferative Neoplasm Progenitors

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INTRODUCTION

Chronic phase (CP) myeloproliferative neoplasms (MPNs), including chronic myeloid leukemia (CML) and myelofibrosis (MF), arise from hematopoietic stem cells. However, they harbor varying propensities to undergo blast crisis (BC; acute leukemic) transformation based on their capacity to give rise to tyrosine kinase inhibitor (TKI) resistant myeloid progenitors (leukemia stem cells; LSC) that activate self-renewal pathways, such as Wnt/β-catenin signaling. Because β-catenin has been reported to regulate human telomerase reverse transcriptase (hTERT), we investigated the capacity of a telomerase complex inhibitor, imetelstat, to prevent malignant progenitor self-renewal. The telomerase complex consists of hTERT, an RNA template subunit (TERC), and a protective shelterin scaffold. Imetelstat is a novel, first in class covalently lipidated 13-mer oligonucleotide telomerase inhibitor with clinical activity in myeloid malignancies. Recent clinical trials showed early signs of efficacy in myeloproliferative neoplasms such as MF. However, the role of imetelstat in selectively eradicating self-renewing MPN progenitors has not been elucidated.

OBJECTIVES

We performed progenitor RNA sequencing (RNA-seq) analysis, stromal co-cultures and humanized MPN progenitor primagraft studies to investigate the ability of imetelstat to selectively inhibit malignant progenitor self-renewal at doses that spare normal progenitors as well as to determine the mechanism of action.

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METHODS and RESULTS

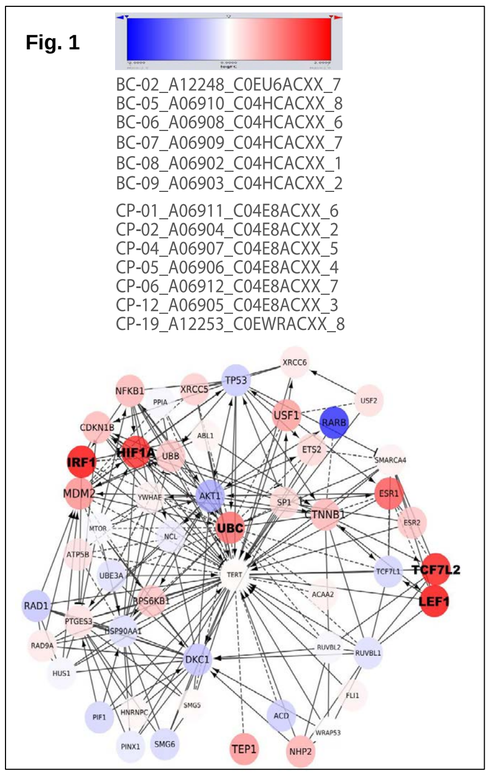

Fig. 1 Cytoscape and Reactome FI 2015 Network Visualization of all genes interacting with TERT. Portraying edgeR statistics in BC (n=6) vs CP (n=7) samples. Each gene must have a minimum CPM of 0.5 in at least 6 samples. The nodes represent genes, and are colored according to edgeR logFC. Red indicates higher expression in BC; Blue indicates higher expression in CP; Colors capped at +/- 2 logFC for pure Red and pure Blue. Bolded text indicates edgeR FDR < 0.10. Text font size is larger as FDR approaches 0.

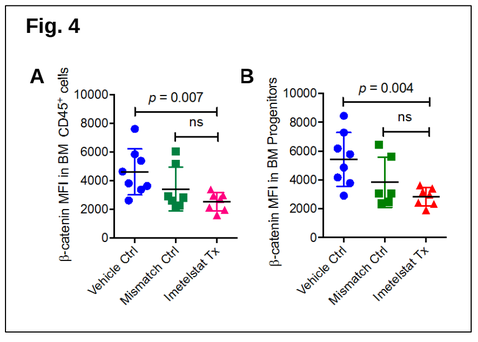

Fig. 4 Imetelstat treatment inhibits β-catenin activity in BM $CD45^+$ cells (A) and BM progenitor cells (B) in BC CML engrafted mouse models (FACS analysis).

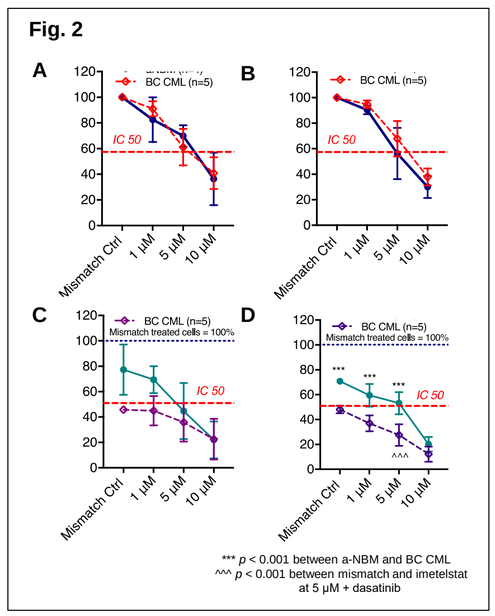

Fig. 2. Imetelstat treatment induces dose-dependent inhibition of survival and self-renewal of BC CML LSC in vitro combined with dasatinib. Human $CD34^+$ cells sorted from aged normal bone marrow (a-NBM) or BC CML patient's samples were co-cultured with SL/M2 stromal cells treated with imetelstat, or combined with dasatinib at 1 nM with imetelstat (1 μM, 5 μM or 10 μM) for 14 days. Cells were collected for qRT-PCR & replating assays. Imetelstat alone induced a dose-dependent inhibition of survival (A) and self-renewal (B) in a-NBM and BC CML. Combination treatment resulted in a significant difference in survival (C) and self-renewal (D) of BC CML while sparing normal progenitors.

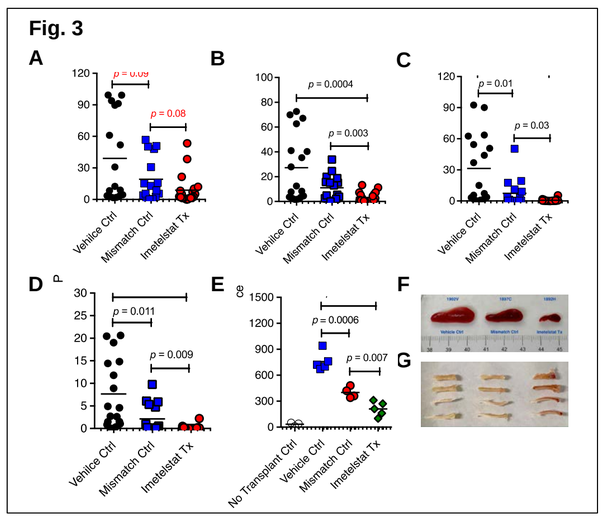

Fig. 3 Imetelstat treatment significantly inhibits $hCD45^+$ & $CD34^+CD38^+$ cells in humanized myeloid BC CML mouse models (n=4). Human $CD34^+$ cells from myeloid BC CML patients were transplanted into $Rag2^{-/-}\gamma c^{-/-}$ mice. Engrafted mouse models were grouped into (1) vehicle, (2) mismatch control, and (3) imetelstat treatment. Bone marrow (BM) and spleen (SP) were harvested for FACS analysis when completing treatments. (A) $hCD45^+$ and (B) Progenitor cells in BM were significantly inhibited after imetelstat treatment, (C) $hCD45^+$ and progenitor cells (D) in SP were significantly inhibited after imetelstat treatment compared to the controls. (E) Spleen size was significantly decreased after imetelstat treatment compared to controls. Imetelstat treated BC CML mouse spleens (F) are smaller, and imetelstat treated BC CML mouse BM (G) normalize in appearance.

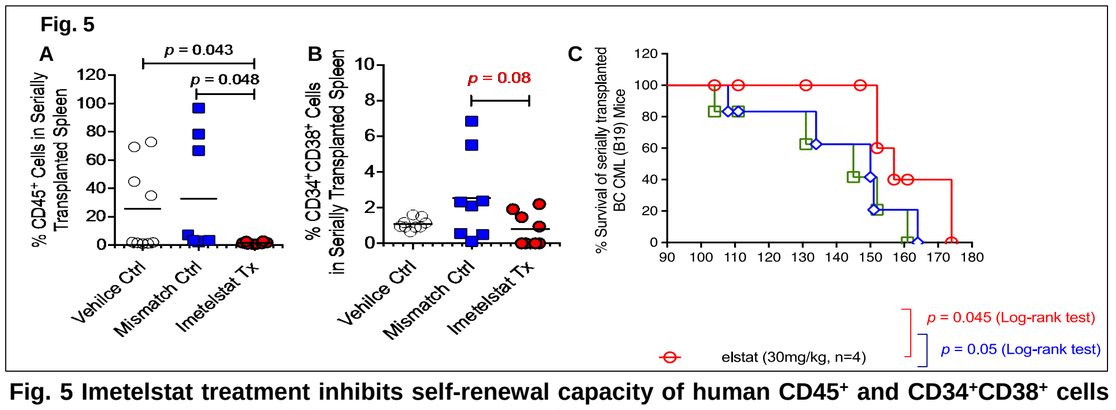

Fig. 5 Imetelstat treatment inhibits self-renewal capacity of human $CD45^+$ and $CD34^+CD38^+$ cells in humanized myeloid BC CML mouse spleens. Self-renewal capacity of both the human $CD45^+$ cells (A) and the progenitor cells (B) were inhibited after imetelstat treatment when compared with the controls. Serially transplanted $Rag2^{-/-}\gamma c^{-/-}$ mice with the $CD34^+$ cells isolated from imetelstat treated mice survived significantly longer than those serially transplanted with $CD34^+$ cells isolated from both vehicle and mismatch control treated mice (C).

CONCLUSIONS

Imetelstat treatment inhibits self-renewal of β-catenin activated BC CML LSC while sparing normal progenitors.