Combination Treatment with Imetelstat, a Telomerase Inhibitor, and Ruxolitinib Depletes Myelofibrosis Hematopoietic Stem Cells and Progenitor Cells

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Abstract

Therapy with imetelstat (Ime), a telomerase inhibitor, has been shown to have disease-modifying effect in patients with myelofibrosis (MF) (Tefferi N Engl J Med 2015; Mascarenhas Blood 2018). This has been attributed to the ability of Ime to selectively deplete MF hematopoietic stem cells (HSC)/progenitor cells (HPC) (Wang Blood Adv 2018). As reported, the primary toxicities of Ime are cytopenias, however, they appear to be reversible, and most importantly, manageable without meaningful clinical consequences (Mascarenhas Blood 2018, Fenaux EHA 2019). Ruxolitinib (Rux) is the first and only JAK 1/2 inhibitor approved for use in patients with intermediate- or high-risk MF. Based on different mechanisms of action for Ime and Rux, we hypothesize that Ime in combination with Rux might create a regimen that would be more efficacious than single agent alone in depleting MF HSCs/HPCs. Using in vitro HPC assays and in vivo HSC assays, we evaluated the therapeutic potential of Rux and Ime.

MF splenic (n=7) or cord blood (CB) (n=3) CD34+ cells (2.5×104/mL) were incubated in serum free expansion medium containing cytokines in the presence of Rux (50nM) alone, Ime alone (1.8uM), Rux+Ime (simultaneous treatment) or Rux→Ime (sequential treatment). Rux was dosed for 3 days and Ime for 14 days under treatment conditions containing either or both drugs. In addition, parallel cultures with mismatched oligonucleotides (MM) or vehicle alone were performed. Compared to either drug alone or to MM control, Rux followed by Ime sequential treatment resulted in significant reductions in the numbers of MF Lin-CD34+ cells (Rux→Ime vs. Rux alone, p=0.001; vs. Ime alone, p=0.059; vs. Rux→MM, p=0.052) and assayable HPCs [(CFUGM+BFU-E+CFU-GEMM), Rux→Ime vs. Rux alone, p=0.02; vs. Ime alone, p=0.05; vs. Rux→MM, p=0.04], while the simultaneous treatment with Rux+Ime did not significantly reduce MF Lin-CD34+ cells and assayable HPCs. By contrast, none of these treatment conditions affected the behavior of normal HSCs/HPCs. Genotyping of individual colonies showed that sequential drug treatment of CD34+ cells from 2 JAK2V617+ MF patients resulted in greater reductions in both the percentage and absolute numbers of JAK2V617F+ myeloid progenitors than that achieved with Ime alone or simultaneous combination treatment. These findings suggest that an additive inhibitory activity against MF malignant HSCs/HPCs can be achieved with sequential treatment of Rux followed by Ime.

We next assessed if treatment with Ime (10mg/kg, 3 times/week for 4 weeks, I.P. injection) and Rux (45mg/kg, daily for 7 days, oral gavage) had additive effects on MF or normal HSCs by directly treating NSG mice transplanted with MF or CB CD34+ cells with these two drugs either alone, in combination simultaneously or sequentially. Mice were sacrificed 4 months after the transplantation. Both simultaneous and sequential drug treatment of mice transplanted with MF splenic CD34+ cells resulted in greater reductions in the degree of human CD45+ marrow cell chimerism (relative to vehicle alone: Rux→Ime: 54.0%; Rux+Ime: 72.4%; Rux alone: 76.6%, Ime alone: 80.0%) and human CD45+ splenic cell chimerism (relative to vehicle alone: Rux→Ime: 13.8%; Rux+Ime: 31.9%; Rux alone: 163.1%; Ime alone: 42.4%) as compared with either drug alone treatment. Similar to our findings in the above in vitro studies, a greater reduction in the absolute number of human CD34+ cells was observed with sequential but not simultaneous combination treatment as compared with either drug alone treatment in the marrow of the mice transplanted with MF CD34+ cells (relative to vehicle alone: Rux→Ime: 36.0%; Rux+Ime: 61.0%; Rux alone: 47.5%, Ime alone: 57.3%). Furthermore, similar results were also observed in depletion of MF long-term HSCs in mice receiving splenic CD34+ cells from an additional patient. However, this same sequential drug schema did not affect normal HSC function. Collectively, these data indicate that alterations of scheduling of the administration of Rux and Ime affect the efficacy of this drug combination in depleting MF HPC/HSCs. We propose that cycles of Rux followed by Ime represents a potentially effective therapeutic strategy that is capable of eliminating MF HSCs/HPCs with an acceptable toxicity profile.

Rationales

Imetelstat

- Has potential disease-modifying activity in patients with myelofibrosis (MF) (Tefferi A, et al. *N Engl J Med.* 2015; 373(10):908-19; Mascarenhas J, et al. *Blood.* 2018; 132 (S1): 685).
- Potentially extends overall survival of patients with intermediate-2 or high-risk MF who have relapsed on or are refractory to JAK inhibitor therapy, including those who are triple negative (Mascarenhas J, et al. *Blood.* 2018; 132 (S1): 685)
- Is capable of selectively depleting malignant MF hematopoietic stem cells (HSC)/progenitor cells (HPC) (Wang X, et al. *Blood Adv.* 2018; 2:2378-2388)
- Leads to cytopenias, however, they appeared to be reversible, and most importantly, manageable without meaningful clinical consequences (Mascarenhas J, et al. *Blood.* 2018; 132 (S1): 685; Fenaux P, et al. *EHA.* 2019)

Ruxolitinib

- Is a small molecule inhibitor of JAK1/2
- Is first FDA approved drug for the treatment of intermediate or high-risk MF
- Is capable of inhibiting but not eliminating *JAK2V617F*+ MF HPCs (Wang X, et al. *Blood.* 2014; 124:2987-95; Manshouri T, et al. *Cancer Sci,* 2008; 99:1265-73)
- Only affects a subpopulation of MF HPCs, while sparing MF HSCs (Wang X, et al. *Blood.* 2014; 124:2987-95)
- May worsen anemia or lead to reversible thrombocytopenia

Hypothesis

Due to imetelstat and ruxolitinib having different mechanisms of action, we hypothesize that a combination of these two drugs might effectively deplete MF HSCs/HPCs

Imetelstat (GRN163L)

A lipid-conjugated 13-mer oligonucleotide complementary to the template region of TERC (the RNA component of telomerase, hTR)

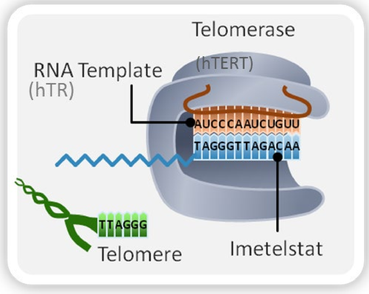
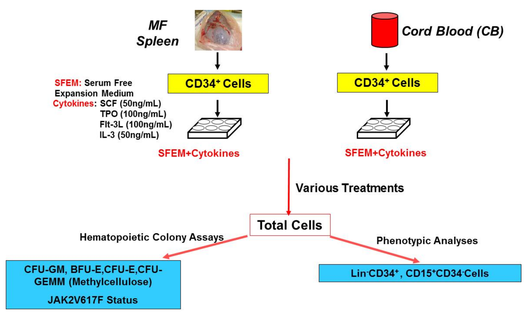
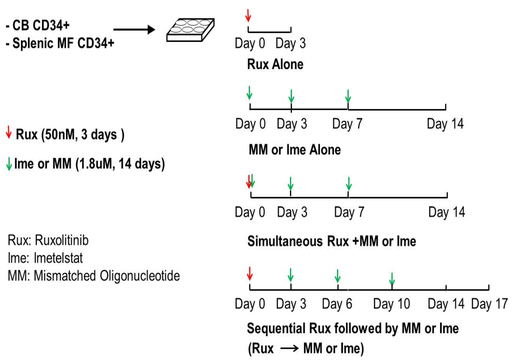


Image Source: Geron

Experimental Design of In Vitro Assay

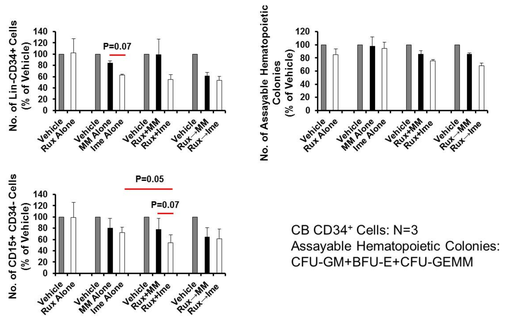


In Vitro Drug Treatment Strategy



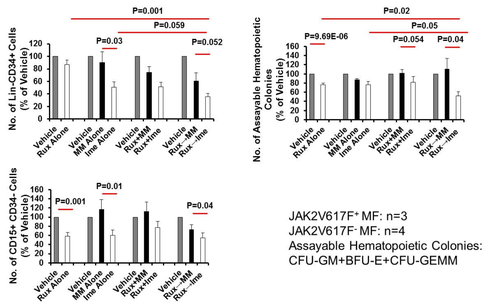
Results

Combination Treatment with Ruxolitinib and Imetelstat Does Not Have Additive Inhibitory Effect on Normal HSCs/HPCs



CB CD34+ Cells: N=3
Assayable Hematopoietic Colonies: CFU-GM+BFU-E+CFU-GEMM

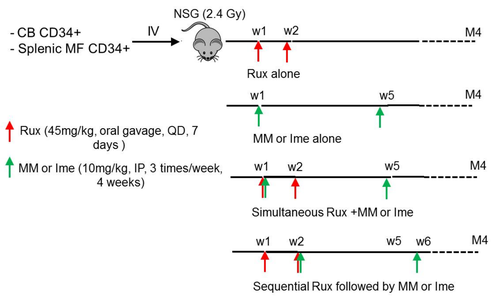
Sequential Treatment with Ruxolitinib Followed by Imetelstat Has An Additive Inhibitory Activity against MF HSCs/HPCs



JAK2V617F+ MF: n=3
JAK2V617F- MF: n=4
Assayable Hematopoietic Colonies: CFU-GM+BFU-E+CFU-GEMM

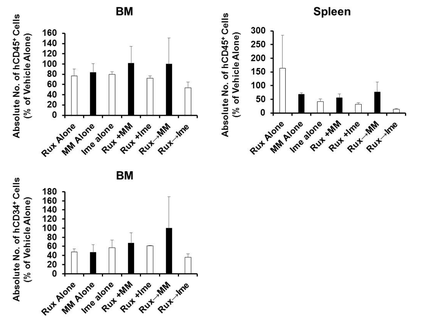
Sequential Treatment with Ruxolitinib Followed by Imetelstat Reduces the Number of JAK2V617F+ Hematopoietic Colonies

Experimental Design of In Vivo Assay

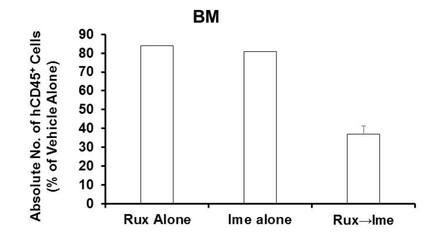


Simultaneous or Sequential Drug Treatment Does not Affect the Behavior of Normal HSCs

Sequential Treatment with Ruxolitinib Followed by Imetelstat Has An Additive Effect on Depleting MF Stem Cells



Treatment with Ruxolitinib Followed by Imetelstat Synergistically Depletes HSCs from A Patient with Triple Negative MF



Summary

- Sequential treatment with ruxolitinib followed by imetelstat has an additive inhibitory activity against MF malignant HSCs/HPCs. By contrast, neither simultaneous nor sequential combination treatment with ruxolitinib and imetelstat affects the behavior of normal HSCs/HPCs.
- Sequential treatment with ruxolitinib followed by imetelstat has an additive effect in depleting MF long-term HSCs assayed using a patient-derived xenograft MF mouse model, However, this same sequential drug schema did not affect normal HSC function.
- Our data indicate that alterations of scheduling of the administration of ruxolitinib and imetelstat affect the efficacy of this drug combination in depleting MF HPC/HSCs.
- These data suggest that ruxolitinib followed by imetelstat represents a potentially effective therapeutic strategy that may be capable of eliminating MF HSCs/HPCs with an acceptable toxicity profile.

Conflict-of-Interest Disclosure

This work was supported by Geron Corporation and Janssen Research & Development LLC.