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14. ABSTRACT
Myeloproliferative neoplasms (MPNs) are blood cancers that affect almost 300,000 people in the United States. While recent drugs (JAK inhibitors) have been developed to treat MPNs these drugs do not effectively induce remission in MPN patients. Thus there is continued need to develop effective therapies for these blood cancers. The purpose of this work is to determine the effect of statins alone and in combination with JAK inhibition on MPN cells driven by the JAK2-V617F oncogene. We have determined that statins have growth inhibitory effects on MPN cells and induce cell death by apoptosis. Addition of mevalonate prevents statin-induced growth inhibition demonstrating the effect is through the intended inhibition of the mevalonate pathway. Statin treatment also inhibited the colony formation of primary cells from MPN patients. Similar statin treatment did not inhibit colony formation of primary cells from healthy individuals. Using an MPN mouse model, statin treatment alone did not affect disease formation. These studies have set up our studies for year two where we will combine statin and JAK inhibitor treatment to study the effects of combination therapy on MPN cells.

15. SUBJECT TERMS
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Introduction

Myeloproliferative neoplasms (MPNs) are blood cancers that affect almost 300,000 people in the United States (1). A mutant protein, JAK2-V617F, is an important factor in the development of these diseases as it is present in the majority of patients with MPNs (2). Targeting this mutant protein has been the goal of much research and recently a JAK inhibitor, ruxolitinib, was approved for use in MPNs. Unfortunately JAK inhibitors like ruxolitinib are unable to significantly reduce the number of diseased cells in patients and thus are unable to offer hope for remission (3). Thus, there is continued need to develop effective therapies for these blood cancers. One approach that has gotten much attention is to develop combination therapies of a JAK inhibitor and another agent. We recently, demonstrated that the cell signaling activity of JAK2-V617F is dependent on lipid rafts in the cell membrane (4). Lipid rafts are small regions of the membrane that are rich in cholesterol and other lipids (5). Depleting cholesterol from the rafts disrupts JAK2-V617F signaling (4). Utilizing a statin to decrease cellular cholesterol, we demonstrated that the growth of MPN cells driven by JAK2-V617F is sensitive to cholesterol-lowering drugs (4). As statins are widely used to control hypercholesterolemia (6), it is possible that combination of a JAK inhibitor with a statin may provide a more efficacious therapy than JAK inhibitor alone.

Body

Task 1 - To determine the extent to which statins can cooperate with JAK2 inhibition in combination therapy of MPN cells. (months 1-18)

In task 1, our studies are designed to determine the extent to which statins can cooperate with JAK2 inhibition in combination. This includes determining the efficacy of statins toward MPN model cell killing and growth and to determine statin effects on growth inhibition and cell killing of primary MPN cells. Our first experiments were designed to determine if there was a statin that was most efficacious against JAK2-V617F-driven cells. We found that simvastatin (Fig. 1), and lovastatin (Fig. 2) each could inhibit the growth of MPN model cells, including HEL and Set2 cells, which are dependent on JAK2-V617F for growth. From this data we have identified simvastatin as the most efficacious statin toward inhibiting the growth of MPN model cells.

Figure 1: Effect of simvastatin on MPN model cell line growth. HEL and Set2 cells were incubated with varying concentrations of simvastatin. Total viable cells were determined by trypan blue over time. Simvastatin exhibited a dose dependent growth inhibitory effect on both cell lines.
Simvastatin reduced cell viability of HEL cells, which are driven by JAK2-V617F, but not K562 cells, which are driven by the BCR-ABL tyrosine kinase (Fig. 3). This suggests there may be some specificity toward which oncogene-driven growth is affected by simvastatin.

We tested if the reduction in cell viability of HEL cells in response to simvastatin was due to induction of apoptosis by performing annexin V binding experiments. These experiments demonstrated an increase in annexin V binding to cells treated with simvastatin compared to controls (Fig. 4). This suggests simvastatin treatment induces apoptosis and this may be a mechanism by which it inhibits HEL cell growth.
Statins inhibit the activity of HMG-CoA Reductase, which results in inhibition of mevalonate synthesis (6). Therefore, in order to determine if the statin effect observed on JAK2-V617F-dependent cells was due to the expected mechanism of HMG-CoA Reductase inhibition we performed mevalonate add-back experiments. Mevalonate add-back completely prevented the simvastatin effects on HEL cell growth (Fig. 5). This demonstrates that the statin effect observed is indeed due to the expected mechanism of action, that is, inhibition of HMG-CoA Reductase.

**Figure 4: Effect of Simvastatin on HEL Cell Apoptosis.** HEL cells were incubated with 1 uM and 5 uM simvastatin. Apoptosis was measured by Annexin V staining followed by flow cytometry after 24 and 48 hours. Apoptosis was detected at the higher simvastatin dose after 48 hours of treatment.

**Figure 5: Mevalonate rescues HEL cells from the growth inhibitory effect of simvastatin.** HEL cells were incubated with 1 uM and 5 uM simvastatin in the presence or absence of 100 uM mevalonate. Total numbers of viable cells were determined by trypan blue exclusion over time. Mevalonate prevented the growth inhibition induced by 5 uM simvastatin suggesting that the mechanism of action of simvastatin is indeed as expected, that is, the inhibition of HMG-CoA reductase.
Myeloid progenitor cells from MPN patients form erythropoietin-independent erythroid colonies in methylcellulose (7). This is a hallmark of these cells and is used as an assay to test new therapies against MPN cells (8). Simvastatin was chosen as the most effective statin to pursue in these studies. Treating primary MPN patients’ cells in this assay reduced the ability of myeloid progenitor cells to form colonies in methylcellulose (Fig. 6). Simvastatin did not affect colony formation from healthy controls. This result is important as it suggests statins may be able to affect aberrant signaling that leads to myeloid cell growth and differentiation in patients.

![Figure 6: Statin treatment inhibits MPN patient progenitor cell colony formation.](image)

**Figure 6**: Statin treatment inhibits MPN patient progenitor cell colony formation. MPN progenitor cells from MPN patients form erythropoietin-independent erythroid colonies in methylcellulose. A. Simvastatin inhibited this colony formation in three JAK2-V617F-positive MPN patients. MPN1 is a polycythemia vera patient and MPN2 and MPN3 are myelofibrosis patients. B. Simvastatin did not inhibit erythropoietin-dependent colony formation in healthy controls. This data suggests that statins may affect the growth properties of neoplastic myeloid progenitor cells in MPN patients and thus may be able to inhibit myeloproliferation in MPN patients.

Task 2 - To determine the extent to which combination therapy of statins and JAK2 inhibition can inhibit MPN formation in a mouse model. (months 4-24)

In task 2, we have performed preliminary studies assessing the effects of statin mono-therapy in an MPN murine model. We initiated therapy 14 days after engraftment at which time mice had severe disease. Statin mono-therapy for 14 days did not result in a significant reduction in the white blood cell count, platelet count, or spleen size compared to animals treated with vehicle control.

Current studies are aimed at 1) assessing the efficacy of longer-term (28 days) therapy and 2) assessing the impact of combination ruxolitinib/statin therapy on disease formation.
**Key Research Accomplishments**

- MPN cells are sensitive to statin treatment.
- Simvastatin is more efficacious, than other statins tested, on growth inhibition of MPN model cells.
- Statin treatment decreases MPN model cell viability.
- Statins induce apoptosis of MPN cells and this may be a mechanism by which they induce growth inhibition.
- The growth inhibition of MPN cells induced by statin treatment is prevented by mevalonate addition, suggesting the mechanism of action of statins against MPN cells is inhibition of HMG-CoA Reductase.
- Erythropoietin-independent growth of myeloid progenitors from MPN patients is inhibited by simvastatin.
- Erythroid colony formation of cells from healthy controls is not inhibited by the same concentration of statin that inhibits colony formation of MPN cells.

**Reportable Outcomes**

- Lori Griner, a Ph.D. graduate student was awarded her Ph.D. degree during the first year of this award.
- Lori Griner received a post-doctoral fellowship opportunity from the National Institutes of Health based on her training that was, in part, supported by this award.
- Data from this project was incorporated into a grant submission to the Leukemia and Lymphoma Society during 2013.

**Conclusion**

We have shown that MPN model cell lines are sensitive to statins with simvastatin being the most efficacious. Statins induce a decrease in total viable cell numbers in growth curve analyses. This is largely due to a decrease in cell viability of the culture. This reduction in cell viability is due to in apoptosis in response to statin treatment. The statin effect on MPN model cells was prevented by the addition of mevalonate. This confirms that the mechanism of action of statins on MPN model cells is due to inhibition of HMG-CoA Reductase, and not some off-target effect. Statin treatment also inhibited the growth of cells from MPN patients. Simvastatin inhibited the growth of primary MPN myeloid progenitor cells forming erythropoietin-independent colonies in methylcellulose. Importantly, simvastatin did not inhibit colony formation of progenitor cells from healthy controls. This suggests statin treatment may affect the neoplastic cell growth of MPN patients and not normal hematopoiesis. This is an important observation for the potential of statins to be incorporated into a therapeutic treatment for MPNs.
References

1. MPN Research Foundation. Learning About MPNs. 2013 11/5/13; http://www.mpnresearchfoundation.org/Prevalence


