Preferentially Targeting the Malignant Hematopoietic Clone in Myeloproliferative Neoplasms (MPN)

Ann Mullally
Dana-Farber/Brigham and Women’s Cancer Center, Harvard Medical School

Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoiesis that arise from the hematopoietic stem cell compartment. The individual MPNs, named for their different phenotypes, have a common molecular basis. The JAK2V617F mutation is present in nearly all patients (>97%) with polycythemia vera (PV) and approximately half of patients with essential thrombocythemia (ET) and myelofibrosis. A small number of patients with ET (1%) and myelofibrosis (5%) have activating mutations in the thrombopoietin receptor, also known as the myeloproliferative leukemia protein (MPL). In addition, approximately 30-40% of patients with ET and myelofibrosis, and the majority of those with nonmutated JAK2, harbor somatic mutations in the endoplasmic reticulum chaperon protein calreticulin (CALR).

The JAK2V617F, MPL, and CALR mutations are typically mutually exclusive in patients with MPNs.

Although JAK inhibitors demonstrate clinical efficacy in MPNs, they do not preferentially target the malignant hematopoietic clone, calling into question their capacity to alter the natural history of MPNs. Using a combination of in vitro model systems, mouse models, and primary MPN samples, we investigated the differential molecular dependencies of Jak2V617F-mutant hematopoietic cells. Although the selective Jak2 inhibitor TG101348 showed promising antitumor activity in a murine model of JAK2V617F-induced PV, clinical drug development was subsequently discontinued due to excess neurotoxicity. Ruxolitinib, an oral JAK1/2 inhibitor, demonstrated a significant reduction in total symptom score compared with placebo in patients with myelofibrosis. JAK1/2 inhibition reduced the median JAK2V617F allelic burden by only 8% after 72 weeks.

Novel therapeutic targets are needed to improve the nonselective results of current JAK2 inhibitors. In a database of clonally-derived mutant and wild-type cells from individual patients, RECQL5 was consistently overexpressed in patients with MPN. RECQL5 is a member of the RECQ family of DNA repair helicases, which also includes RECQL1, BLM, WRN, and RECQL4. RECQL5 appears to function in the stabilization of stalled replication forks to prevent fork collapse and double-strand DNA breaks. JAK2V617F has been shown to stimulate replication stress and DNA damage. However, the role of RECQL5 as a novel regulator of genome stability in MPNs had not been previously explored. The first step in this new line of investigation involved generated Jak2V617F-myeloid progenitor cell lines. As observed in the patient cell samples, the Jak2V617F mouse cells demonstrated differential expression of Recql5, with normal expression of all other members of the RECQ family. A range of experiments knocking down and inhibiting JAK2 and STAT5 revealed RECQL5 as a downstream target of JAK2-PI3K signaling. RECQL5 depletion sensitized V617F cells to both endogenous and exogenous replication stress. Using rescue experiments verified the on-target effect and key role of RECQL5 in increasing the sensitivity of JAK2V617F+ cells to replication stressors. Similar analyses using peripheral blood samples from MPN patients confirmed these findings, with RECQL5 depletion increasing the sensitivity of JAK2V617F+ cells from patients with myelofibrosis to hydroxyurea.

A mechanistic model of RECQL5-dependent protection against replication stress has emerged. RECQL5 appears to act at the point of replication fork stalling to allow the fork to recover and stabilize. In the absence of RECQL5,
replication fork stalling instead leads to fork collapse and double-stranded break. RECQL5 depletion increases hydroxyurea-induced fork stalling and double-strand break formation. Replication stress-associated cytotoxicity can be amplified specifically in JAK2V617F-mutant hematopoietic cells through RECQL5-targeted synthetic lethality.

**Summary**

Results to date show that RECQL5 is a downstream target of JAK2-P13K signaling. RECQL5 functions to protect JAK2V617F cells from endogenous and exogenous replication stress by regulating replication fork stability. The depletion of RECQL5 sensitizes JAK2V617F cells to pharmacological inducers of replication stress. Our goal is to translate our findings into novel therapeutic approaches aimed at eliminating the malignant hematopoietic clone in MPNs.

**References**

5. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation specifically in JAK2V617F-mutant hematopoietic cells from endogenous and exogenous replication stress by regulating replication fork stability. The depletion of RECQL5 sensitizes JAK2V617F cells to pharmacological inducers of replication stress. Our goal is to translate our findings into novel therapeutic approaches aimed at eliminating the malignant hematopoietic clone in MPNs.

**Table 2. JAK2 Inhibition and Reduction of JAK2V617F Allelic Burden**

<table>
<thead>
<tr>
<th>JAK2V617F Allelic Burden</th>
<th>Ruxolitinib</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean change, baseline to 24 weeks</td>
<td>-10.9%</td>
<td>3.5%</td>
</tr>
<tr>
<td>Mean change, baseline to 48 weeks</td>
<td>-21.5%</td>
<td>6.3%</td>
</tr>
</tbody>
</table>

**Financial Disclosures**

Dr. Mullally discloses no financial relationships relevant to the content of this presentation.

**Acknowledgements**

This summary was created from the proceedings of the 2015 Chabner Colloquium: Collaboration in Clinical Trials, which was held on Monday, October 26, 2015, in Boston, MA. The Society for Translational Oncology received educational grants in support of this activity from AbbVie Inc., Chugai Academy for Advanced Oncology (CHAAO), Epizyme, Inc., Incyte Corporation, Lilly USA, LLC, Merrimack Pharmaceuticals, Inc., Novartis Pharmaceuticals Corporation, Otsuka America Pharmaceutical, Inc., and Pfizer Inc.

© Society for Translational Oncology 2016