Discovery and development of JAK inhibitors for the treatment of myeloproliferative neoplasms

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Discovery and development of JAK inhibitors for the treatment of myeloproliferative neoplasms

Outline

- Discovery of JAK inhibitors
- Preclinical profile of ruxolitinib
- Testing of ruxolitinib in a JAK2\textsuperscript{V617F}-driven model of MPN
- Preclinical combination of ruxolitinib with panobinostat
- Assessment of ruxolitinib effects on cytokines in MF
- Conclusions and Outlook
Towards the identification and development of JAK inhibitors

HT screens → Leads → Medicinal chemistry → Selectivity & safety profiling

JAK enzymatic assays

JAK-dependent cell-based assays

In vivo pharmacology (mice & rats)

In vivo efficacy (mouse mech/disease models)

In vitro & vivo toxicology / DMPK / pre-formulation

Drug candidate, selection for proof of concept in man

number of compounds

Leads
Towards the identification and development of JAK inhibitors

Preclinical profile of ruxolitinib – *in vitro* activity

- Structure, model of binding to the kinase domain, and activity of ruxolitinib in biochemical and cellular assays:

**A**

![Structure of ruxolitinib](image)

**B**

![Model of binding](image)

**C**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>IC$_{50}$ Mean ± SD (nM), at 1 mM ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK1</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>JAK2</td>
<td>2.8 ± 1.2</td>
</tr>
<tr>
<td>JAK3</td>
<td>428 ± 243</td>
</tr>
<tr>
<td>TYK2</td>
<td>19 ± 3.2</td>
</tr>
</tbody>
</table>

**D**

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Ba/F3-EpoR-JAK2$^{V617F}$ cell proliferation assay</th>
<th>pJAK2/pSTAT5/pERK (Ba/F3-JAK2$^{V617F}$ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$</td>
<td>127 ± 17 nM (mean ± SD)</td>
<td>128-320 nM</td>
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</table>

Ruxolitinib demonstrated >100-fold selectivity against a broad panel of kinases

Preclinical profile of ruxolitinib – *in vivo* activity

- Activity in Ba/F3-JAK2^{V617F}- driven mouse model of leukemic disease:

![Graphs and images showing survival, spleen weight, spleen size, and cytokine levels](linked_images)

Testing of ruxolitinib in a murine model of myeloproliferative neoplasm-like disease

- Principle of preclinical bone marrow transduction/transplantation (BMT) models of hematopoietic malignancies:

  Evrot E. et al, Manuscript in preparation
Testing of ruxolitinib in a murine model of myeloproliferative neoplasm-like disease

- Polycythemia and splenomegaly with strongly increased levels of P-STAT5 in mice transplanted with bone marrow expressing JAK2<sup>V617F</sup>:

<table>
<thead>
<tr>
<th></th>
<th>IRES-GFP</th>
<th>mJAK2&lt;sup&gt;V617F&lt;/sup&gt;-IRES-GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT (%)</td>
<td>48.3 ± 0.9</td>
<td>68.6 ± 2.2*</td>
</tr>
<tr>
<td>Retic count (x10&lt;sup&gt;12&lt;/sup&gt;/L)</td>
<td>0.58 ± 0.05</td>
<td>1.26 ± 0.08*</td>
</tr>
<tr>
<td>WBC count (x10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>4.99 ± 0.29</td>
<td>32.69 ± 9.59*</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM, n = 5/group. *p < 0.001 by t-test.
Testing of ruxolitinib in a murine model of myeloproliferative neoplasm-like disease

Evrot E. et al, *Manuscript in preparation*
Testing of ruxolitinib in a murine model of myeloproliferative neoplasm-like disease

- Ruxolitinib suppressed JAK2^{V617F}-driven splenomegaly, while being well tolerated:

![Graph showing spleen weight and body weight changes](image)

*\( p < 0.05 \), ANOVA followed by Dunnett’s test, \( n = 8 \)/group

--- average value for spleen weights in non-transplanted mice

Evrot E. et al, *Manuscript in preparation*
Testing of ruxolitinib in a murine model of myeloproliferative neoplasm-like disease

- Ruxolitinib normalized spleen weight, reticulocyte and WBC counts (3 wks), and reduced GFP-positive circulating cells in lysed blood (2 wks):

Evrot E. et al, Manuscript in preparation
Ruxolitinib treatment markedly suppressed splenic extramedullary hematopoiesis and bone marrow hypercellularity:

Evrot E. et al, Manuscript in preparation
Testing of ruxolitinib in a murine model of myeloproliferative neoplasm-like disease

- P-STAT5 IHC staining on spleen and bone marrow sections:
  - Spleen
    - Vehicle, 2 h
    - 90 mg/kg RUX, 2 h
  - Sternum

Evrot E. et al, *Manuscript in preparation*
Testing of ruxolitinib in a murine model of myeloproliferative neoplasm-like disease

- Reticulin staining on bone marrow sections (representative images from vehicle treated animal and animals in ruxolitinib 60 mg/kg group):

Evrot E. et al, *Manuscript in preparation*
Combination of ruxolitinib with the pan-DAC inhibitor panobinostat in the JAK2\textsuperscript{V617F}-driven MPN model

Evrot E. et al, Manuscript in preparation
Discovery of the JAK2^{V617F} mutation and time until entry of JAK inhibitors into clinical trials

- Rapid entry of JAK inhibitors into clinical trials in MF following the discovery of the JAK2 V617F mutation:
Randomized study of ruxolitinib compared with best available therapy (BAT) in myelofibrosis

- **COMFORT-II** – A randomized, open-label, multicenter phase III trial:

  Patients with MF (N=219)
  
  Randomized 2:1
  
  **Ruxolitinib (oral)**
  
  15 mg or 20 mg bid
  
  **Best available therapy**

Adapted from Harrison C. N. et al, *NEJM*, 2012
Effect of ruxolitinib therapy on cytokines in MF

- Aberrant cytokine levels and JAK1 activation in MF:

Effect of ruxolitinib therapy on cytokines in MF

- Impact of ruxolitinib on inflammatory cytokines:

Adapted from Verstovsek S. et al, *NEJM*, 2010
The objective of this analysis was to evaluate the associations between cytokine levels and spleen size reductions in COMFORT-II

**Spleen Assessment**
- Spleen volume was measured by MRI (or CT scan in appropriate patients) every 12 weeks and spleen length measured by palpation at each study visit

**Plasma Biomarker Assay**
- Plasma samples were analyzed using Rules Based Medicines Human MAP®v1.6
- 89 cytokines were measured at baseline and weeks 4, 24, and 48
- Cytokine markers with > 30% below the lower limit of quantification (LLOQ) at all available visits were not used in analysis (31 out of 89 markers are removed by this criterion)
- For the remaining 58 cytokines,
  - If the cytokine level was less than LLOQ, then the established level was \((0.5) \times \text{LLOQ}\)
  - If both baseline and post-baseline cytokine data were below LLOQ, then the post-baseline data was not established and recognized as missing
Ruxolitinib therapy reduces the levels of many cytokines compared to BAT at weeks 4, 24, and 48.
In the ruxolitinib arm, there was a positive association between the decrease in TNF-α levels from baseline and spleen volume reduction ≥ 35% from baseline at weeks 24 (P<0.01) and 48 (P<0.01), irrespective of JAK2 V617F mutational status.

A 0.59 and 0.61 fold decrease in TNF-α was associated with higher rates of spleen response (volume reduction ≥ 35% from baseline) at weeks 24 (P=0.02) and 48 (P=0.01), respectively.
Role of TNF-α in myeloproliferative neoplasms

TNFα facilitates clonal expansion of JAK2V617F positive cells in myeloproliferative neoplasms

Angela G. Fleischman,1 Karl J. Aichberger,1,2 Samuel B. Luty,1 Thomas G. Bumm,1 Curtis L. Petersen,1 Shirin Doratotaj,3 Kevin B. Vasudevan,1 Dorian H. LaTocha,1 Fei Yang,4 Richard D. Press,4 Marc M. Loriaux,1,4 Heike L. Pahl,5 Richard T. Silver,6 Anupriya Agarwal,1 Thomas O’Hare,7 Brian J. Druker,1,8 Grover C. Bagby,1,9 and Michael W. Deininger7

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TNF-α selects for JAK2V617F+ colony formation
Association of change in leptin levels with spleen volume reduction in all ruxolitinib treated patients by mutation status

- Ruxolitinib-treated patients with the largest increases in leptin from baseline to weeks 24 and 48 also had greater reductions in spleen volume at weeks 24 (P<0.01) and 48 (P<0.01), irrespective of JAK2 V617F mutational status.

- A 2.67 and 2.02 fold increase in leptin was associated with higher rate of spleen response at weeks 24 (P=0.11) and 48 (P=0.01), respectively.

Harrison C.N. et al, ASCO, 2012
Conclusions and Outlook

- Ruxolitinib demonstrates efficacy in preclinical models of JAK2\textsuperscript{V617F}-driven models of MPN-like diseases

- In MF patients, ruxolitinib provides significant improvement in splenomegaly and MF-associated symptoms, and results in changes in plasma cytokine levels

- In the ruxolitinib arm, several cytokines exhibited significant reductions that were not observed in the BAT arm and were consistent across patients

- It is likely that the reduction in the levels of a number of these cytokines, either individually or together result in the resolution of many of the symptoms observed on ruxolitinib therapy

- Irrespective of JAK2 V617F mutational status, changes in TNF-\lowercase{α} and leptin were associated with reductions in spleen size at week 24 and 48, for patients treated with ruxolitinib, but not for those treated with BAT

- Although further analyses must be performed, these data indicate that TNF-\lowercase{α} and leptin may provide value in predicting and analyzing the response to ruxolitinib therapy
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- Srdan Verstovsek
- Tiziano Barbui

**Participating Patients**
Association of the change in leptin levels and weight gain in patients by treatment group

- Increases in leptin levels from baseline to weeks 24 and 48 were observed in the ruxolitinib arm and this increase preceded and was associated with weight gain at weeks 24 and 48

Harrison C.N. et al, ASCO, 2012
Role of OSM in myeloproliferative neoplasms

V617F-dependent amplifier of cytokine production and bone marrow remodeling in myeloproliferative neoplasms

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JAK2V617F induces expression of OSM

JAK2 inhibition reduces OSM in SET-2 cells

Detection of OSM in MPN patient BM