LSD1 Inhibitor CPI-482 Shows Efficacy and Prolongs Survival in Mouse Models of AML and Post-MPN AML in the Context of Constitutive JAK-STAT Pathway Activation

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Disclosures

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McGrath: Constellation Pharmaceuticals: Current Employment, Current equity holder in publicly-traded company.


Wang: Constellation Pharmaceuticals: Current Employment, Current equity holder in publicly-traded company. Levine: Imago: Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; C4 Therapeutics: Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; Isoplexis: Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; Celgene: Consultancy, Honoraria, Research Funding; Roche: Consultancy, Honoraria, Research Funding; Lilly: Consultancy, Honoraria; Janssen: Consultancy; Astellas: Consultancy; Morphosys: Consultancy; Novartis: Consultancy; Amgen: Honoraria; Gilead: Honoraria; Prelude Therapeutics: Research Funding; Qiagen: Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; Loxo: Current equity holder in private company, Membership on an entity’s Board of Directors or advisory committees. Trojer: Constellation Pharmaceuticals: Current Employment, Current equity holder in publicly-traded company.
CPI-482 is a potent LSD1 inhibitor with a novel MOI

- CPI-482 is a novel, highly potent styrenylcyclopropylamine-based LSD1 inhibitor

- Hit triage utilized a suite of biochemical and cell-based screening assays:
  - Histone demethylase activity: TR-FRET
  - LSD1:Histone H3 interaction: Nano-BRET assay
  - Cell-based target engagement: Quantigene LY96 assay
  - Phenotypic assay: Kasumi-1 cell proliferation

- CPI-482 modifies the FAD cofactor on LSD1, forming a mixture of meta-stable intermediates which uniquely show pH-dependent reversal of this covalent modification

- A highly potent initial reversible binding ($K_i$) component contributes to efficiency of FAD modification by CPI-482

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_{\text{inact}}$ (min$^{-1}$)</th>
<th>$K_i$ (nM)</th>
<th>$k_{\text{inact}} / K_i$ (M$^{-1}$ · S$^{-1}$)</th>
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<tr>
<td>CPI-482</td>
<td>0.15</td>
<td>5.8</td>
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CPI-482 induced differentiation and cell death of leukemia cells

- LSD1 functions as a histone H3 lysine demethylase and a scaffolding protein, assembling into repressive transcriptional complexes.
- CPI-482 inhibits the demethylase activity of LSD1 and disrupts its interaction with co-repressors, resulting in eviction off chromatin.
- Displacement of the LSD1 complex allows for transcriptional reprogramming directed toward differentiation of leukemic cells.

LSD1 complex evicted off chromatin by LSD1 inhibitor CPI-482.

- HDAC
- GFI1B
- PU.1
- CEBPα
- H3K9Ac
- H3K27Ac
- H3K4Me
- mRNAs

Differentiation
Growth suppression
Apoptosis

Leukemic Blasts
Monocytes
CD86

Chromatin-bound LSD1 complex

Repressed

+ CPI-482

Expressed
Activated JAK-STAT signaling correlated with sensitivity to LSD1 inhibition

A short-term (3-day) proliferation assay identified four cell lines with marked sensitivity.

- All had activated JAK-STAT signaling, either due to JAK2V617F mutations or other genomic alterations
- All were derived from AML patients with either antecedent MDS/MPN or displayed cytogenetic hallmarks (5q-/20q-) of MDS/AML

- Two of the AML responder cell lines were explored further using in vivo tumor xenograft models
- A once weekly dosing schedule of CPI-482 resulted in significant tumor growth inhibition of the JAK2V617F mutant SET-2 and HEL 92.1.7 mouse xenograft models.
Anti-leukemogenic gene expression changes elicited by CPI-482

- CPI-482 treatment elicited nearly identical gene expression changes in the two $JAK2^{V617F}$ mutant AML cell lines
- Up-regulated genes parallel those observed with knockdown of the leukemogenic HoxA9 transcription factor
- MYC target genes are uniformly down-regulated by CPI-482 in both HEL and SET-2 cells
CPI-482 showed enhanced efficacy in a \( \text{Jak}^{2V617F} \) post-MPN AML model

**Post-MPN secondary AML model**

- **Bone marrow harvest**
- **c-Kit selection**
- **Retroviral transduction with \( \text{Jak}^{2V617F} \) or empty vector (Migr1)**
- **Injection into lethally irradiated recipients**

**Vehicle**
- Ruxolitinib 60 mg/kg, BID (5on/2off)

**CPI-482**
- 60 mg/kg, QW

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**Model characterization:**
- Mice engrafted with \( \text{Jak}^{V617F}/\text{Tp53}^{\text{null}} \) HSCs show effacement of bone marrow, spleen and liver with myeloblasts.
- Further analysis revealed expansion of c-Kit+ cells with a marked increase of megakaryocyte and erythroid progenitors (MEP).

**Vehicle vs. CPI-482:**
- p<0.001

**Ruxolitinib vs. CPI-482:**
- p<0.043

- Weekly oral dosing with CPI-482 at 60 mg/kg prolonged survival greater than that seen with ruxolitinib, and had no impact on body weight.
- Spleen weight, evidence of extramedullary hematopoiesis, showed a much greater reduction with CPI-482.
CPI-482 improved organ architecture in $\text{Jak}^{2^{\text{V617F}}}/\text{Tp53}^{\text{null}}$ AML model

- Reduction of extramedullary hematopoiesis evident in both spleen and liver following treatment with CPI-482
- In the spleen, CPI-482 treatment improved normal tissue architecture, with clearly defined white and red pulp.
- A marked increase in megakaryocytes and marked reduction in myeloid cells was noted in the spleen of CPI-482 treated mice
- In spleen (as well as bone marrow and liver), evidence of greater reduction in reticulin fibrosis was observed in CPI-482 treated animals compared to ruxolitinib treated animals
CPI-482 stabilized peripheral blood cell populations

- A trend toward lower WBC in CPI-482 treated mice was noted versus vehicle or ruxolitinib treated mice.
- No differences in Hgb between treatment arms was observed.
- A proportion of mice treated with CPI-482 developed decrease in platelet count, an on-target effect of LSD1 inhibition.
Treatment with CPI-482, but not ruxolitinib, was associated with a significant increase in the proportion of lineage positive population in the spleen and bone marrow of treated mice.

Increases in the B-cell, T-cell and Gr1/CD11B populations were noted in the spleen and bone marrow of CPI-482 treated mice.
Conclusion

• CPI-482 is a potent, selective LSD1 inhibitor that demonstrates effective single agent activity in AML cell lines and xenograft models, particularly in the context of JAK-STAT pathway activation

• CPI-482 resulted in transcriptional reprogramming in tumors and up-regulated genes normally repressed by the leukemogenic transcription factor HoxA9, and resulted in down-regulation of Myc target genes

• CPI-482 was more effective than ruxolitinib in an aggressive post-MPN sAML mouse model with $Jak2^{V617F}$ and $Tp53$ loss

• In the sAML mouse model, CPI-482 prolonged survival, reduced spleen size and improved bone marrow architecture and function

• These data support further investigation of a potential therapeutic impact of the LSD1 inhibitor CPI-482 in adverse risk myeloid malignancies, especially in the context of $JAK2^{V617F}$ mutations