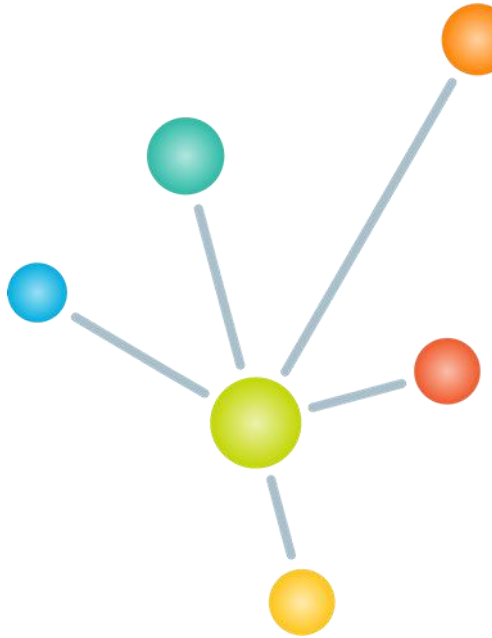
The BET Inhibitor, CPI-0610, Promotes Myeloid Differentiation in Myelofibrosis Patient Bone Marrow and Peripheral CD34+ Hematopoietic Stem Cells



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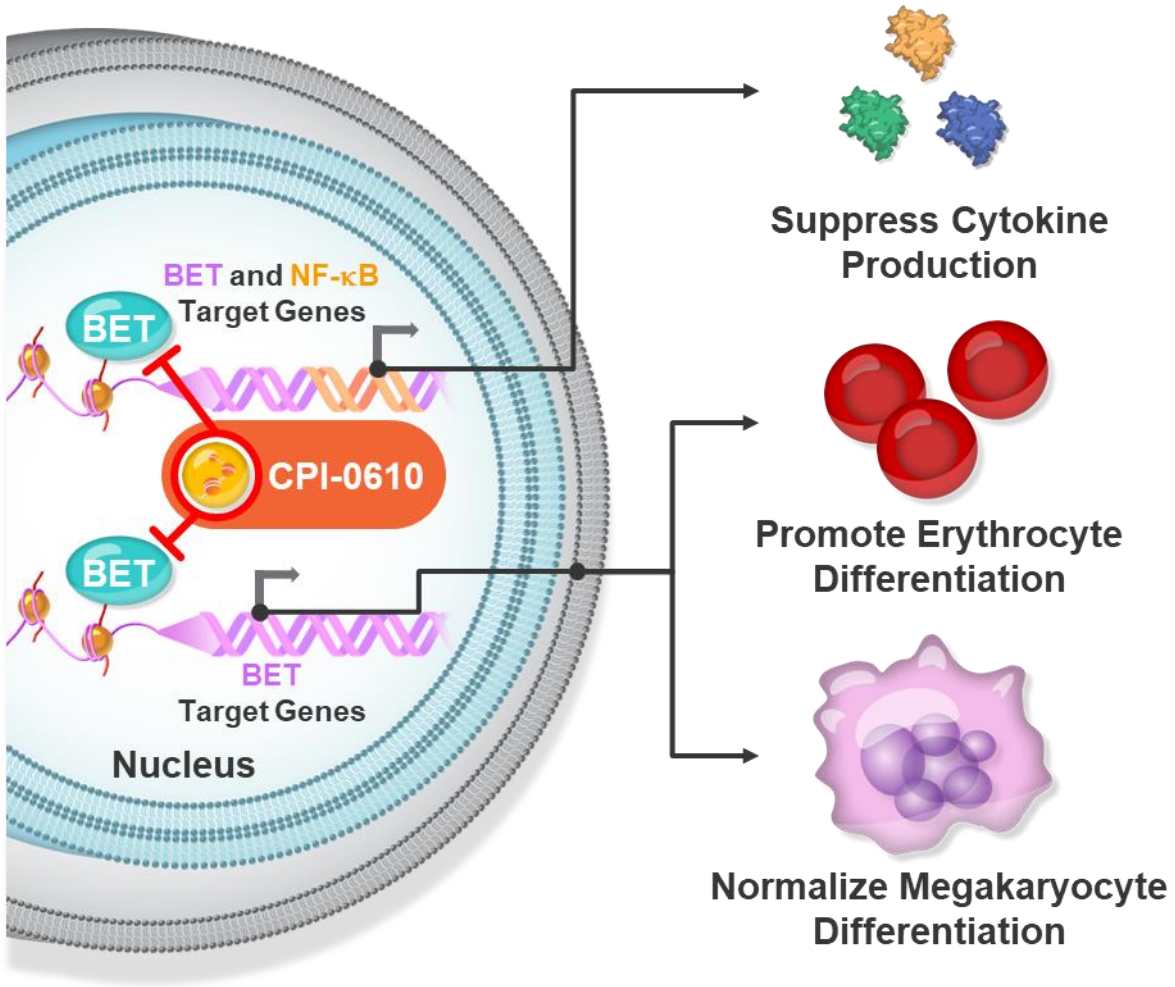
Disclosure Slide

Mohamed E Salama, MD

- Nothing to disclose

- Discussion of off-label drug use: Not applicable

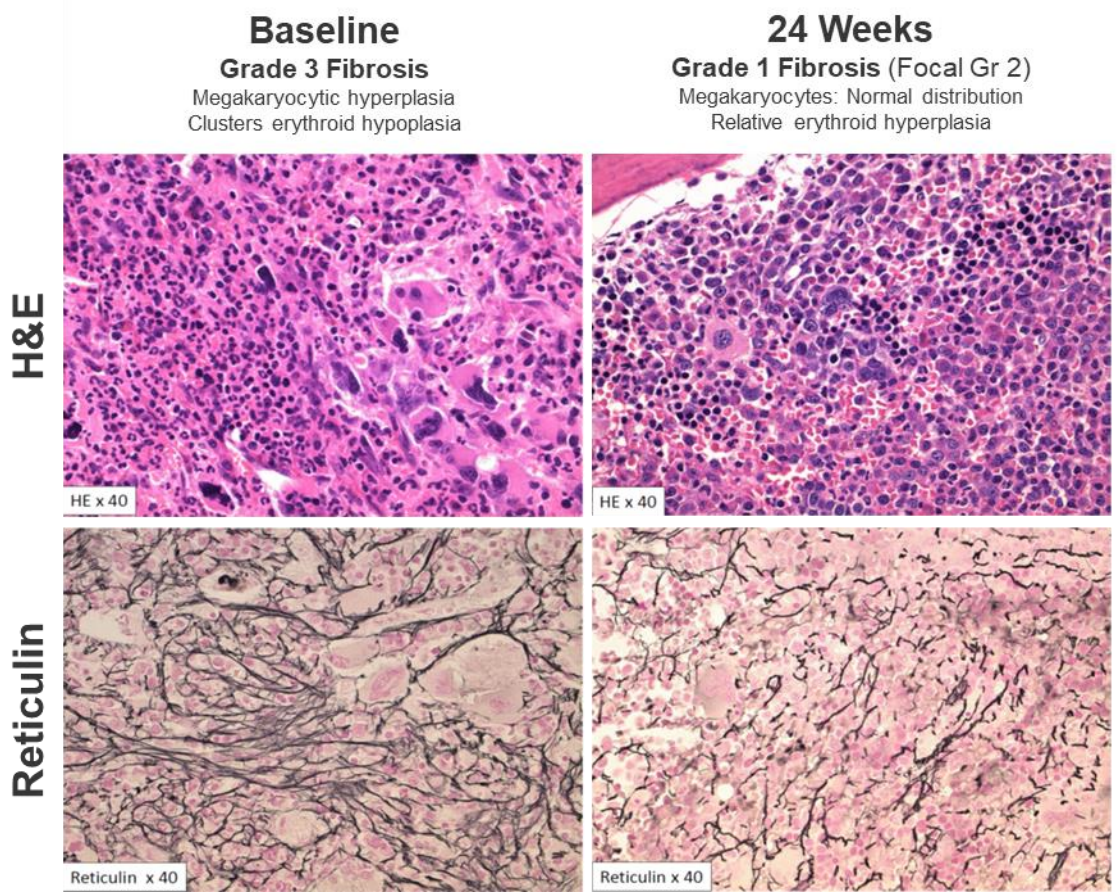
Background: CPI-0610 Improves Hemoglobin Levels & Transfusion Dependency in MF Patients



- Myelofibrosis (MF) is characterized by progressive bone marrow (BM) fibrosis resulting from aberrant megakaryopoeisis and expression of pro-inflammatory cytokines, both of which are heavily influenced by BET mediated gene regulation, leading to myeloproliferation and cytopenias
- CPI-0610 is a potent and selective BET inhibitor under investigation in MF patients as monotherapy or in combination with ruxolitinib in the ongoing MANIFEST trial [NCT02158858]
- In ruxolitinib naïve or experienced MF patients, treatment with CPI-0610 monotherapy or in combination with ruxolitinib not only reduced spleen volume and symptoms, but also improved hemoglobin levels, reduced BM fibrosis, and in some patients overcame transfusion dependency

Local Analyses of MANIFEST Patient Samples Show Improvements in BM Fibrosis

Bone Marrow Fibrosis Grading at Baseline and after 24 Weeks (Arm 3)



Patients evaluable if they have had a baseline and at least one post-baseline bone marrow biopsy by the data cutoff date or discontinued prior to week 24 due to any reason.

Data assessed by local labs per the European consensus on grading bone marrow fibrosis and assessment of cellularity (Thiele J et al., *Haematologica*, 2005,90:1128).

Data cutoff: 29 Sept 2020

Local Pathological Bone Marrow Biopsy Review

- **Arm 1 (CPI-0610 monotherapy in heavily pretreated patients) –** Poster Presentation 2163, Dec 6 10:00 AM-6:30 PM EST
  - **21%** (6/29) of patients showed at least one grade improvement in bone marrow fibrosis
  - 2 patients had worsening
- **Arm 2 (CPI-0610 "add on" to Ruxolitinib in patients with suboptimal response to Ruxolitinib) –** Oral Presentation 0056, Dec 5 11:45 AM EST
  - **41%** (16/39) of patients had at least one grade improvement in bone marrow fibrosis
  - 3 patients had worsening
- **Arm 3 (CPI-0610 + Ruxolitinib in JAK-naïve) –** Oral Presentation 0055, Dec 5 11:30 AM EST
  - **33%** (16/48) of patients showed at least one grade improvement in bone marrow fibrosis
  - **88%** (14/16) improvements occurred within 6 months of treatment
  - 2 patients had worsening

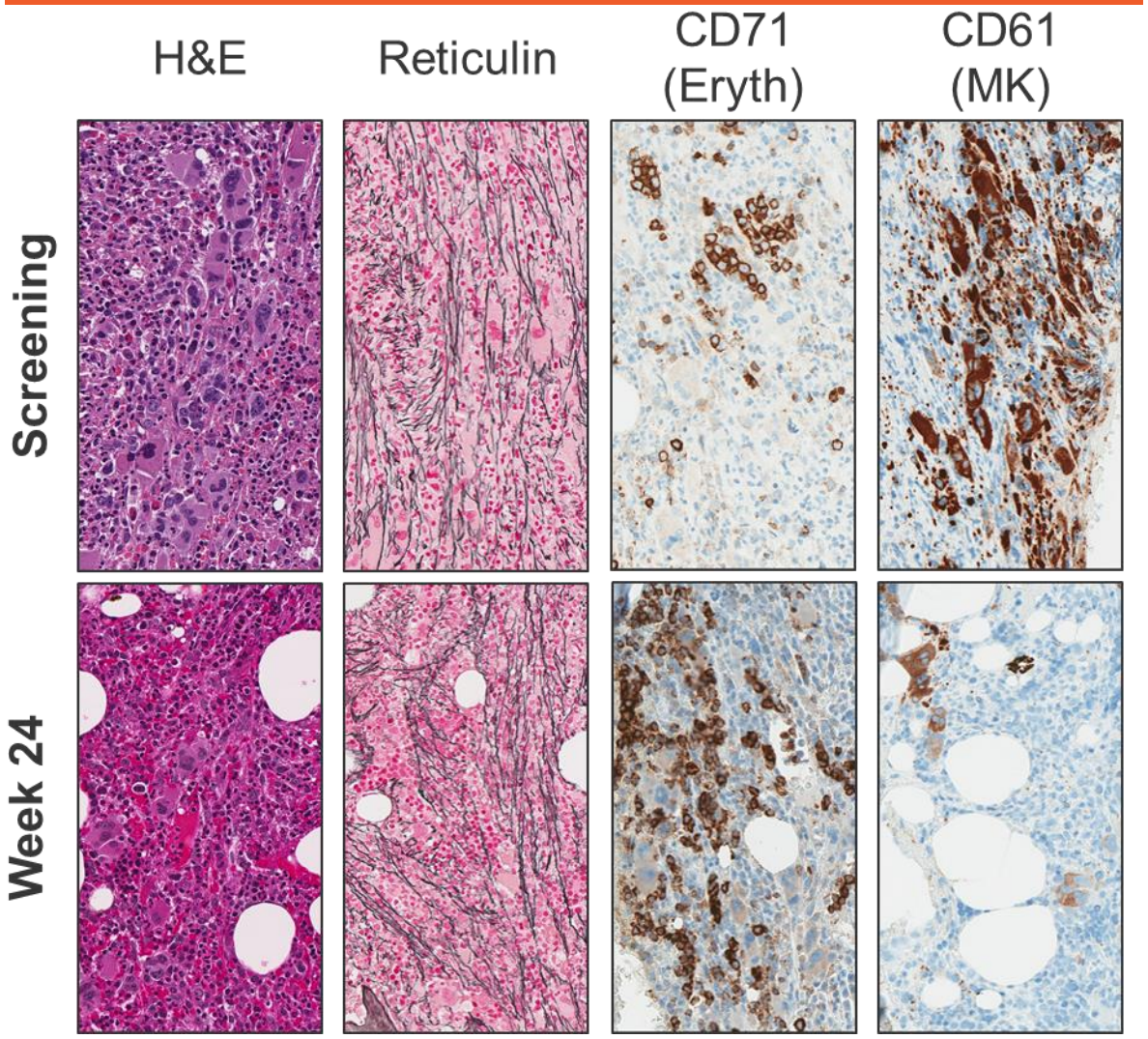
Exploratory Analyses of MANIFEST Patient Samples Show BM Improvements After 24 Weeks of Treatment

Patients with Improvements in Exploratory BM IHC*

Cohort	Erythroid (Eryth)	Megakaryocyte (MK)	Cohort	Reticulin (≥1 Gr Improve)
Arm 1 (n=8)	38%	38%	Arm 1 (n=7)	29%
Arm 2 (n=17)	65%	59%	Arm 2 (n=14)	43%
Arm 3 (n=12)	67%	92%	Arm 3 (n=11)	46%
Total (n=37)	59%	65%	Total (n=32)	41%

*~10 fields per slide evaluated to determine IHC improvement, if allowed by sample size

IHC Analysis from Arm 3 Patient



Improvements in Multiple Markers of BM Biology Observed in the Majority of Patients

Each row represents one patient

- Total of 37 pairs of BM biopsy samples are available from MANIFEST patients for exploratory BM biopsy IHC studies
- BM improvement in erythroid precursors, MK histotopography and reticulin tend to co-occur in MANIFEST patients
- Digital pathology analysis ongoing

Patients with BM Erythroid Improvements Are More Likely to Achieve Transfusion Independence

BM Eryth Improvement at 24W by IHC	TD →TI	Time TI Achieved
		19W
		21W
		33W
		30W
		41W
		14W, 73W

Improvement/conversion

No improvement/conversion

Each row represents one patient

In Arm 2 (CPI-0610 "add on" to ruxolitinib after suboptimal response of MF progression)

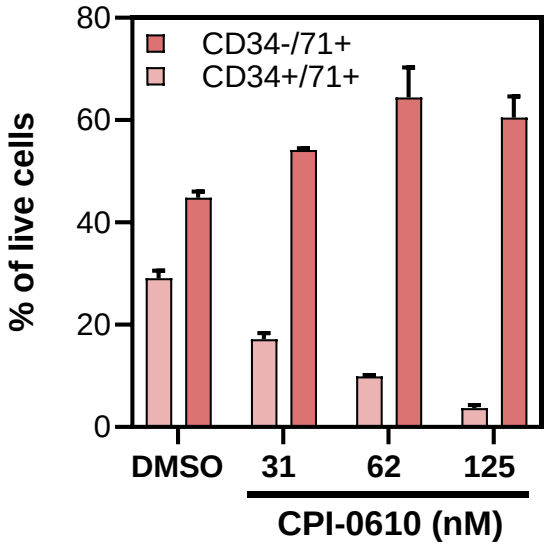
- 12 transfusion dependent (TD) patients provided BM biopsy sample pairs (baseline & 24 week) for IHC analysis
  - 8/12 showed erythroid improvement in BM IHC
  - 4/12 showed no change in BM erythroid IHC
- 6 of these 12 TD patients become transfusion independent (TI)
  - 5/8 patients with erythroid improvement converted from TD to TI
  - 1/4 patients without erythroid improvement converted from TD to TI

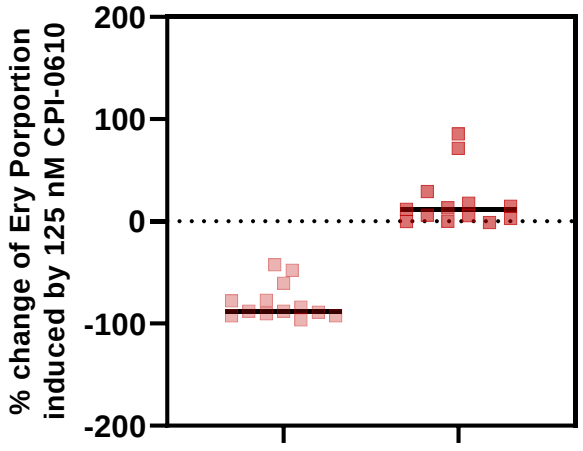
CPI-0610 Promotes Maturation of Erythroid Progenitors Ex Vivo

- CPI-0610 promotes erythroid maturation of CD34+ cells by:
  - Inducing maturation from CD34+/CD71+ to CD34-/CD71+ populations

    Or
  - Increasing the proportion of the CD71+/CD235ab+ population (see next slide)

CD34 and CD71 populations

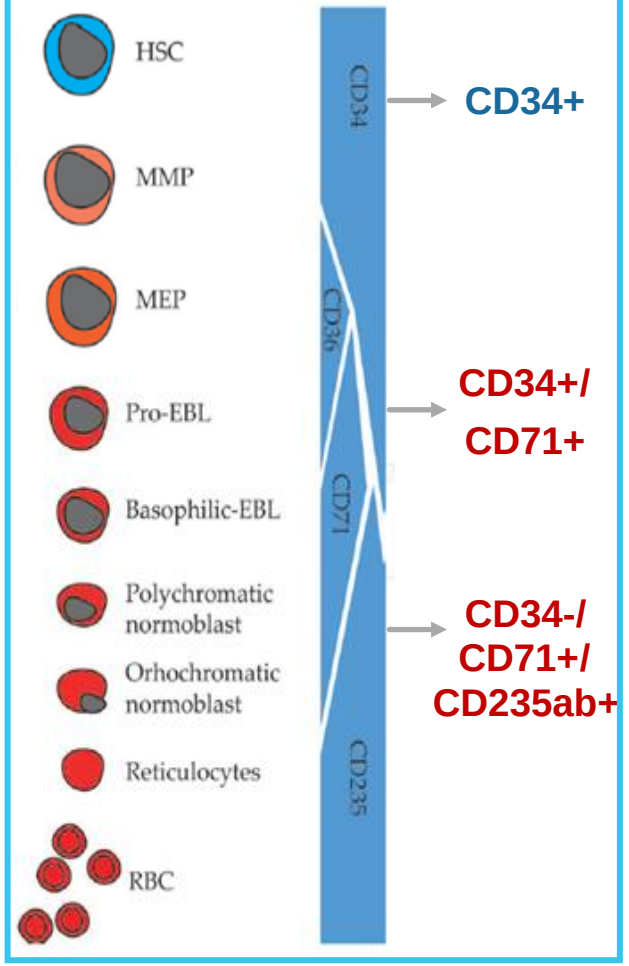




Each dot represent 1 MF patient

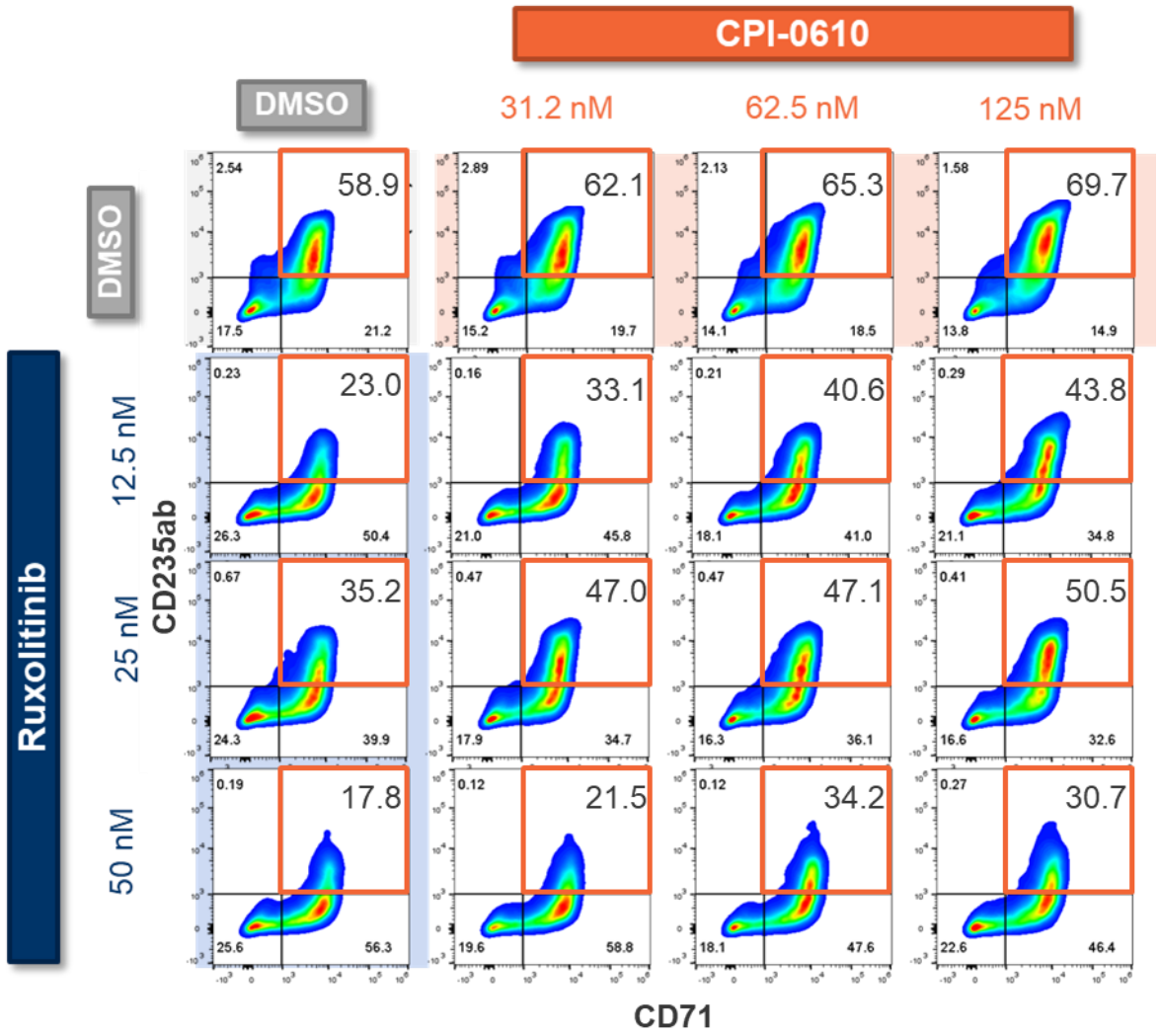
CD34+ cells isolated from MF patient blood samples at baseline and differentiated in vitro for 7 days in presence of 125 nM CPI-0610 using cytokine cocktail of SCF, IL3 and EPO

Erythroid Cell Surface Marker Expression During Erythroid Lineage Commitment

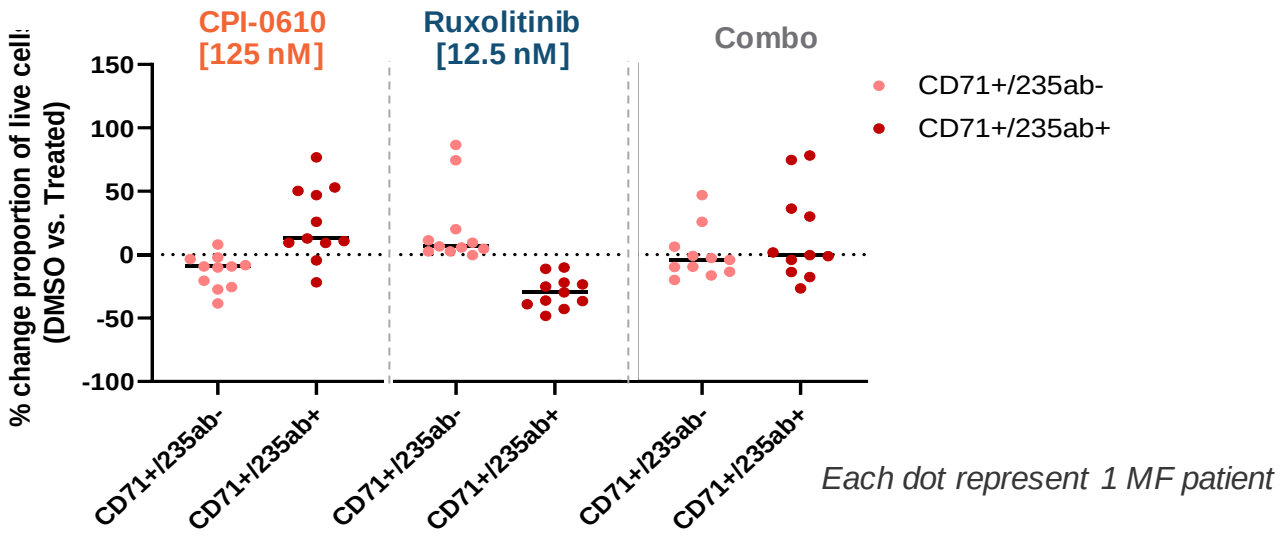


Adapted from Varga et al 2018, IntechOpen chapter

CPI-0610 Partially Reverses Ruxolitinib-Mediated Suppression of Erythroid Differentiation Ex Vivo



- CPI-0610 promotes erythroid maturation at clinically-relevant exposures
- Ruxolitinib suppresses erythroid differentiation & proliferation at clinically-relevant exposures
- CPI-0610 partially reverses ruxolitinib-mediated suppression of erythroid differentiation

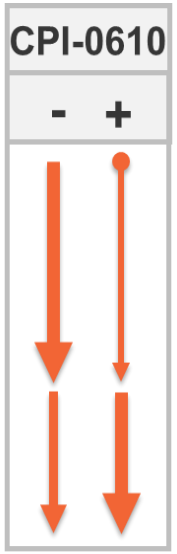


Each dot represent 1 MF patient

CD34+ cells isolated from MF patient blood samples at baseline and differentiated in vitro for 7 days in presence of 31.2 nM, 62.5 nM or 125 nM CPI-0610, 12.5 nM, 25 nM or 50 nM ruxolitinib or combination as indicated using cytokine cocktail of SCF, IL3 and EPO

CPI-0610 Inhibits MK Proliferation, Promotes Maturation of Late MK Cells Ex Vivo

- CPI-0610 treatment inhibits MK proliferation in a dose dependent manner
- CPI-0610 treatment increase proportion of more mature **late MK** cells, while reducing the proportion of **early MK** cells



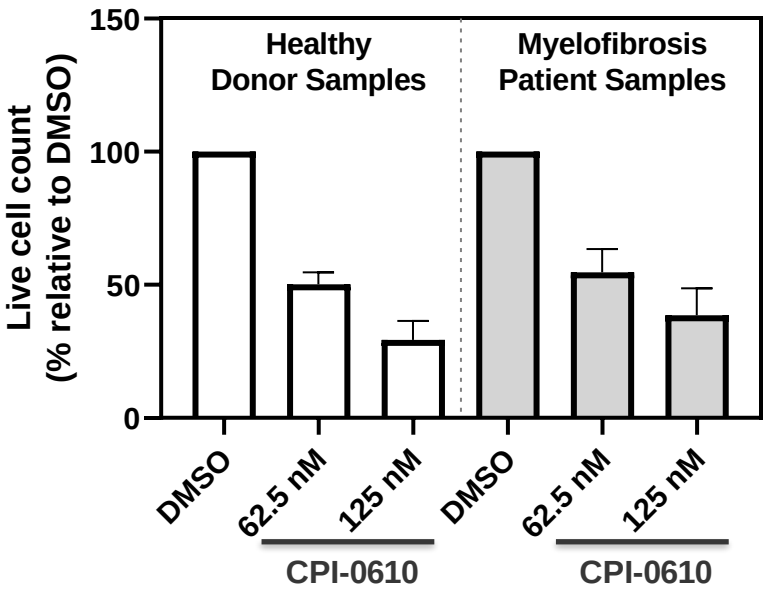
@ day 7 MK differentiation

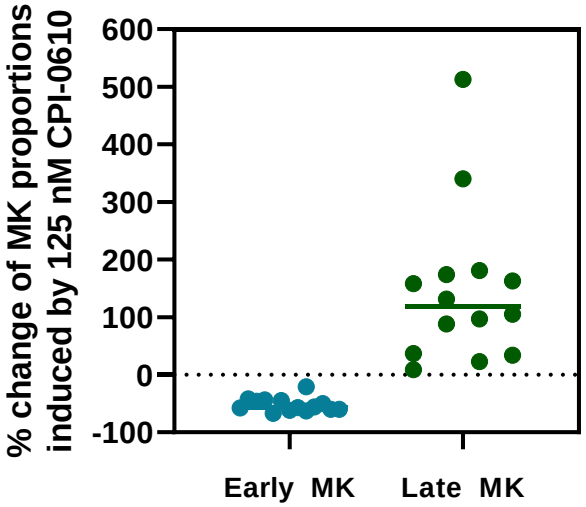
$CD34^{+}CD41a^{-}CD42b^{-}$ **Prog/non-MK**

$CD34^{+}CD41a^{+}CD42b^{-}$ **MK blast**

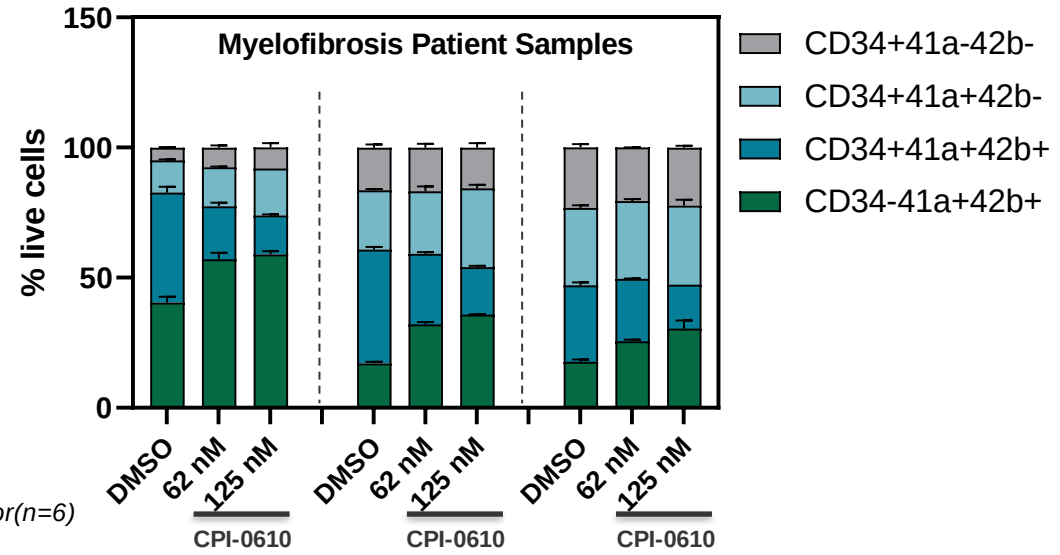
$CD34^{+}CD41a^{+}CD42b^{+}$ **Early MK**

$CD34^{-}CD41a^{+}CD42b^{+}$ **Late MK**





Each dot represents 1 MF patient (n=8) or healthy donor(n=6)



CD34+ cells isolated from MF patient blood at baseline and differentiated in vitro for 7 days in presence of 62.5 nM or 125 nM CPI-0610 using cytokine cocktail of SCF, IL6, IL9 and TPO

Conclusions

- Bone marrow (BM) fibrosis grading by local labs of 116 MANIFEST patient baseline versus on treatment sample pairs suggests a relative reduction in BM fibrosis severity
  - 32.8% (38/116) ≥1 grade improvement, 6% (7/116) ≥1 grade worsening (across all 3 arms)
- Exploratory independent review of erythroid and megakaryocytic markers in 37 MANIFEST patient BM biopsy sample pairs (baseline versus 24 week on treatment) suggests relative improvement in BM function
  - Increased erythroid progenitors
  - Improvements in histotopography of MK cells
  - Decreases in reticulin fibers
- Ex vivo differentiation of MF patient CD34+ cells from baseline peripheral blood samples shows that CPI-0610 promotes maturation of MK and erythroid progenitors and can partially reverse ruxolitinib-mediated suppression of erythroid differentiation
- These preliminary BM and PBMC ex vivo data from MANIFEST patients suggest that CPI-0610 may have a disease-modifying effect in myelofibrosis