Identification of Small Molecules that Selectively Target JAK2^{V617F} Driven Cytokine-Independent Megakaryopoiesis by Leveraging Single Cell RNA Sequencing Maps of Myelofibrosis Patients Samples and a Deep Learning Framework

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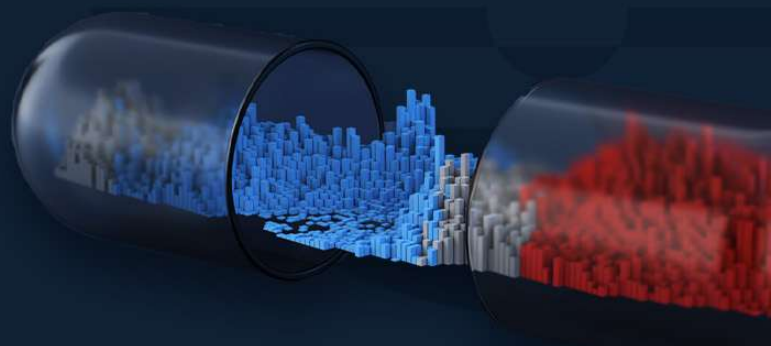
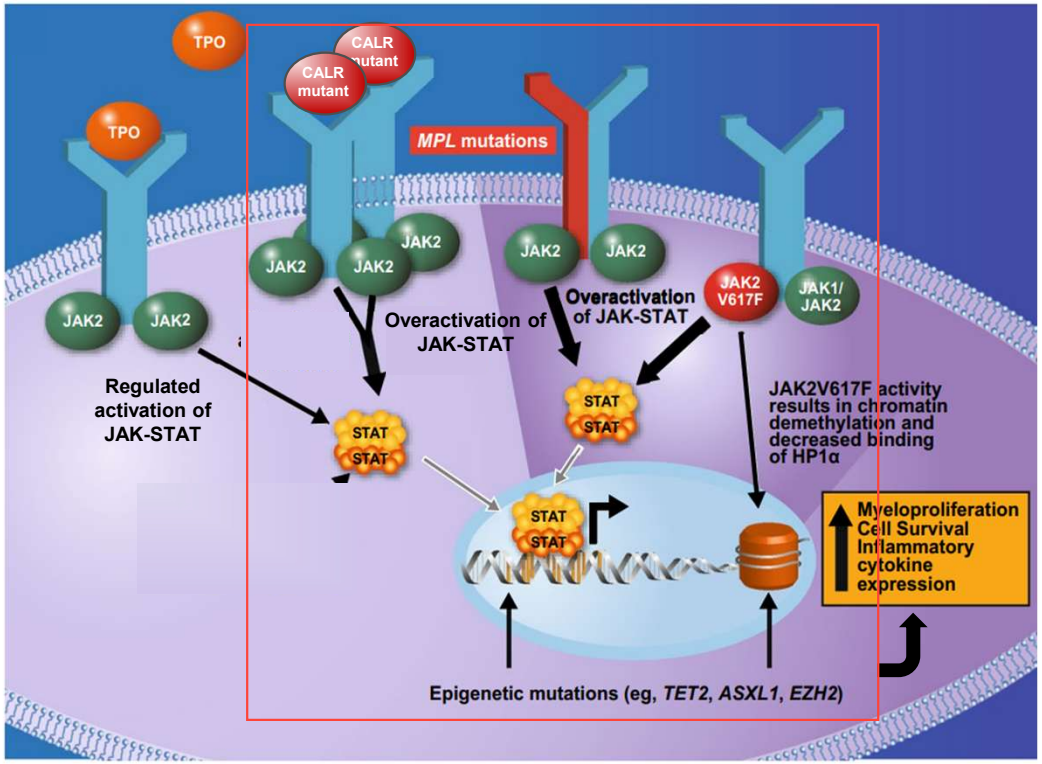


Myeloproliferative neoplasms are driven by dysregulation of JAK2 signaling



Adapted from Bryan et.al 2016

- Myelofibrosis is a myeloproliferative neoplasm marked by bone marrow fibrosis, cytopenias, inflammation, and splenomegaly
- Myelofibrosis is a progressive disease resulting in bone marrow failure or transformation to leukemia
- Somatic mutations in the hematopoietic stem cell compartment result in the **overactivation** of JAK2 signaling resulting in the myeloproliferative phenotype
- Current approved drugs manage the disease symptoms, but are not disease modifying
- There is a need to develop selective therapies that target the root cause of the disease

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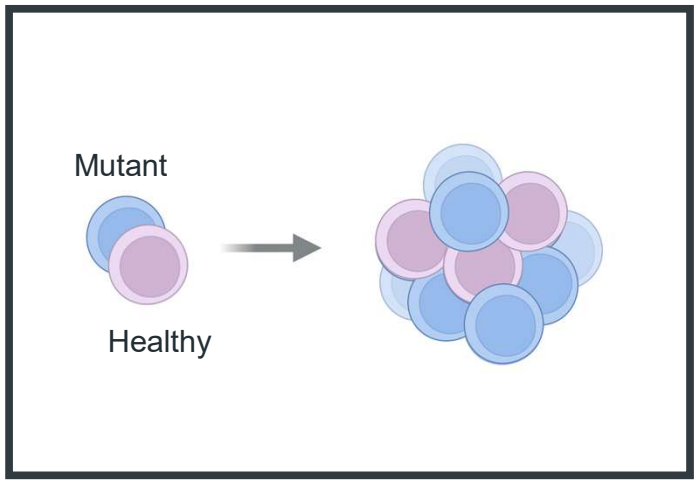
Selectively targeting the mutant clone through transcriptomics



Targeting the **Hematopoietic stem and progenitor cell (HSPC)** compartment

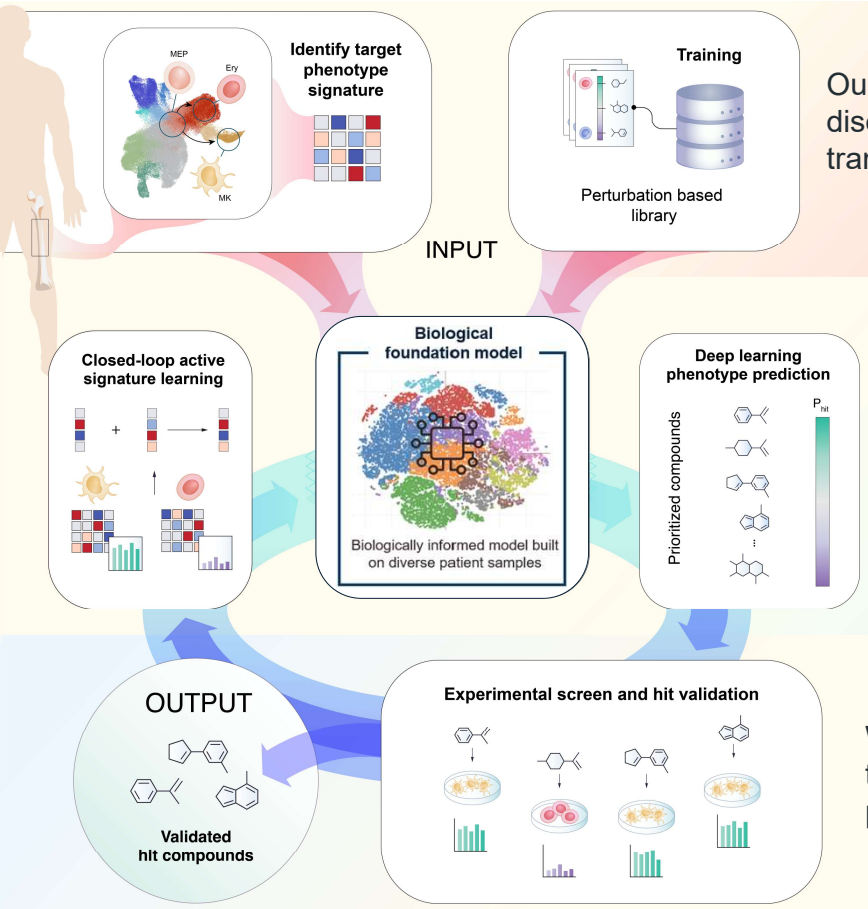
- Hyperactivity of the JAK-STAT signaling pathway is the central hallmark of MPNs
- There is accumulating evidence that the **JAK2 ^{V617F} HSPCs have unique epigenetic profiles** resulting in cell-intrinsic inflammatory signatures compared to WT HSPCs
- We hypothesized that there is a **unique transcriptional program in mutant JAK2 HSPCs** that can be targeted**,** resulting in selective inhibition of the mutant clone, while sparing the healthy cells

Our Discovery Platform leverages disease atlases and deep learning to connect disease biology to chemical matter through transcriptomics

We build a **clinical single cell atlas** to identify cell transitions and associated transcriptional signatures

We link **transcription to phenotype** establishing causality and enabling lead optimization

We identify **chemical structures regulating our phenotypes** of interest



Our **foundation model** connects disease biology to chemistry through transcriptomics

Our models **predicts chemical perturbations** that induce a desired transcriptional change

We **test predictions in vitro** for their ability to induce/revert a given **phenotype**

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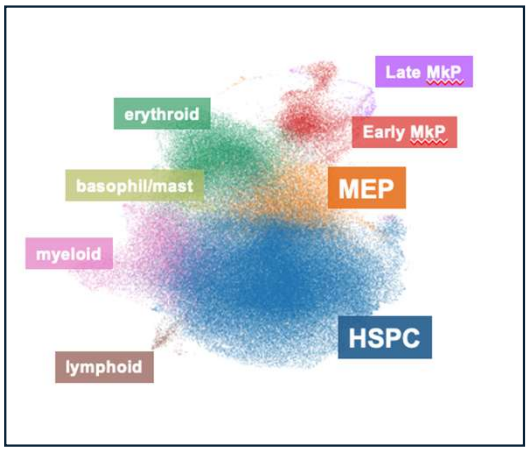
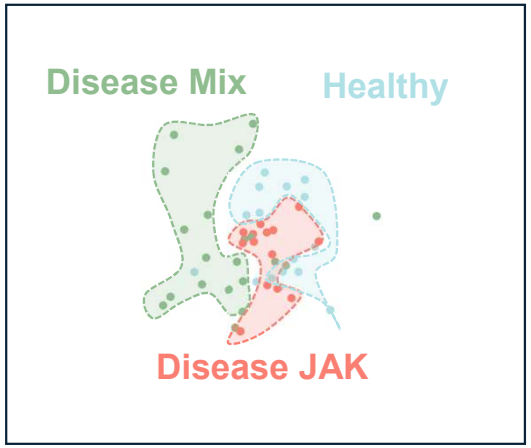
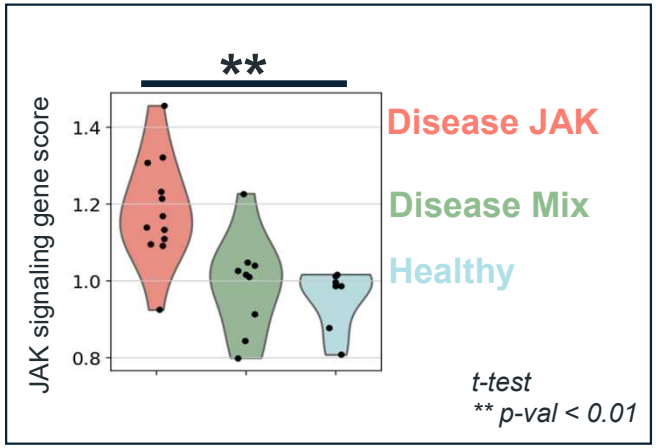
A single cell atlas of myelofibrosis allows to investigate cell state transitions in distinct cellular compartments



Built MF disease atlas
196,428 cells, 50 patient samples
using public and internal data



Donor stratification based on transcriptomic profile in HSPC compartment



Unbiased cell clustering identified a sub-group of MF patients with increased JAK activity signature in HSPC compartment

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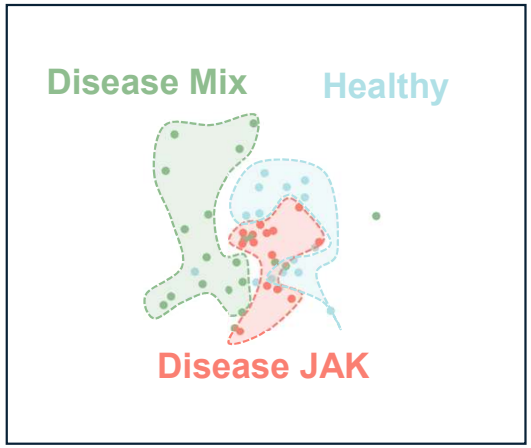
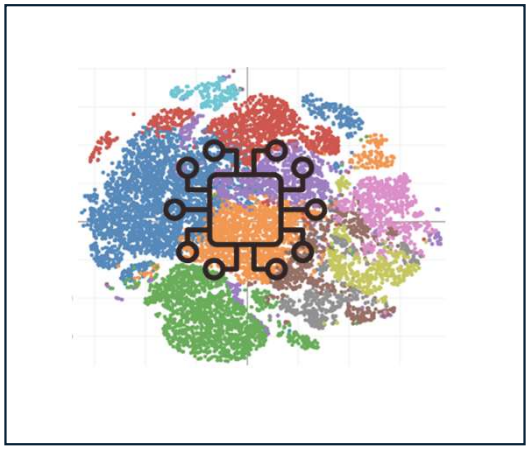
Leveraging our disease atlas and deep learning framework we predicted interventions targeting the HSPC compartment

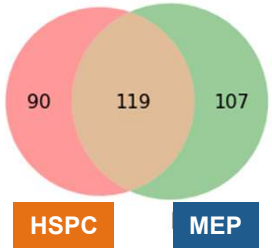


Disease JAK to Healthy was used as our cell state transition within HSPC and MEP compartments



Our deep model and proprietary perturbation library was deployed to make in silico predictions

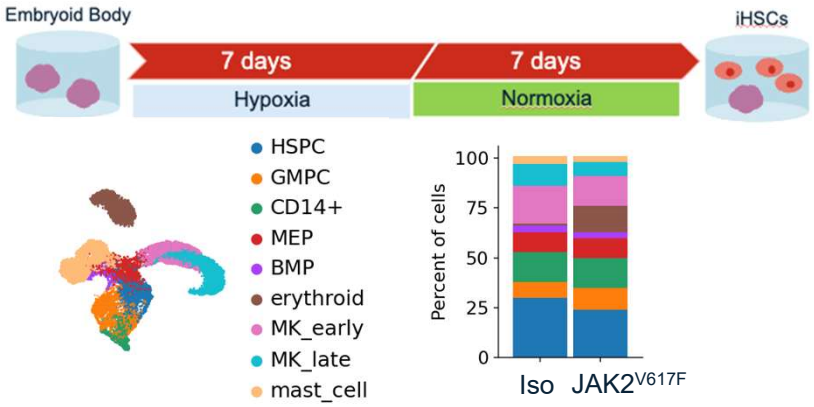
ML Predictions	HSPC	MEP
Cellarity Deep Model permutation p<0.05	209	226

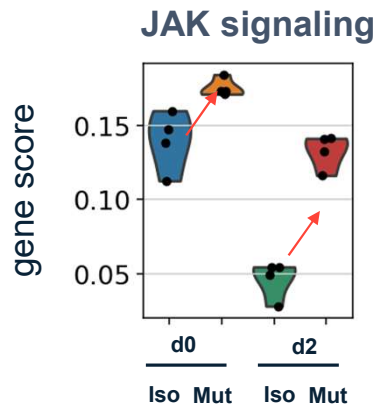


Predictions were unique to cellular compartment and covered diverse MOAs

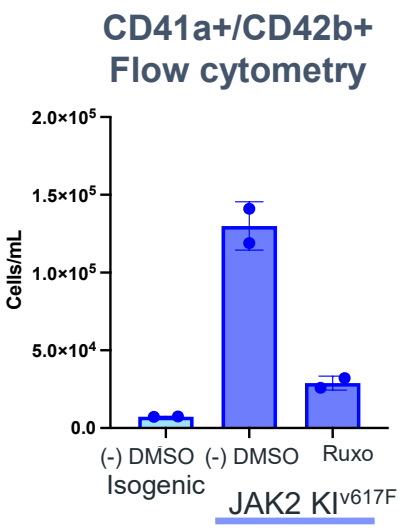
Ruxolitinib and other JAK targeting compounds were also predicted

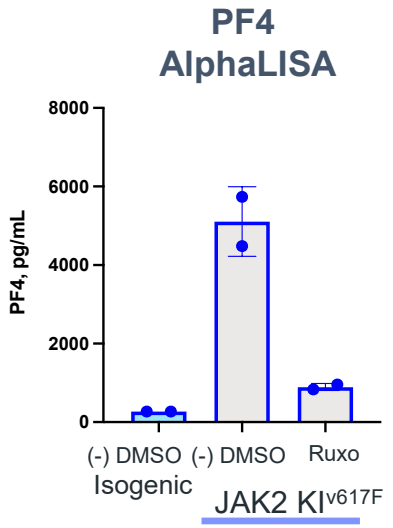
iHSCs harboring the JAK2^{V617F} mutation captures disease cell states from clinical atlas and functional endpoints of myelofibrosis





TPO Independent Megakaryopoiesis

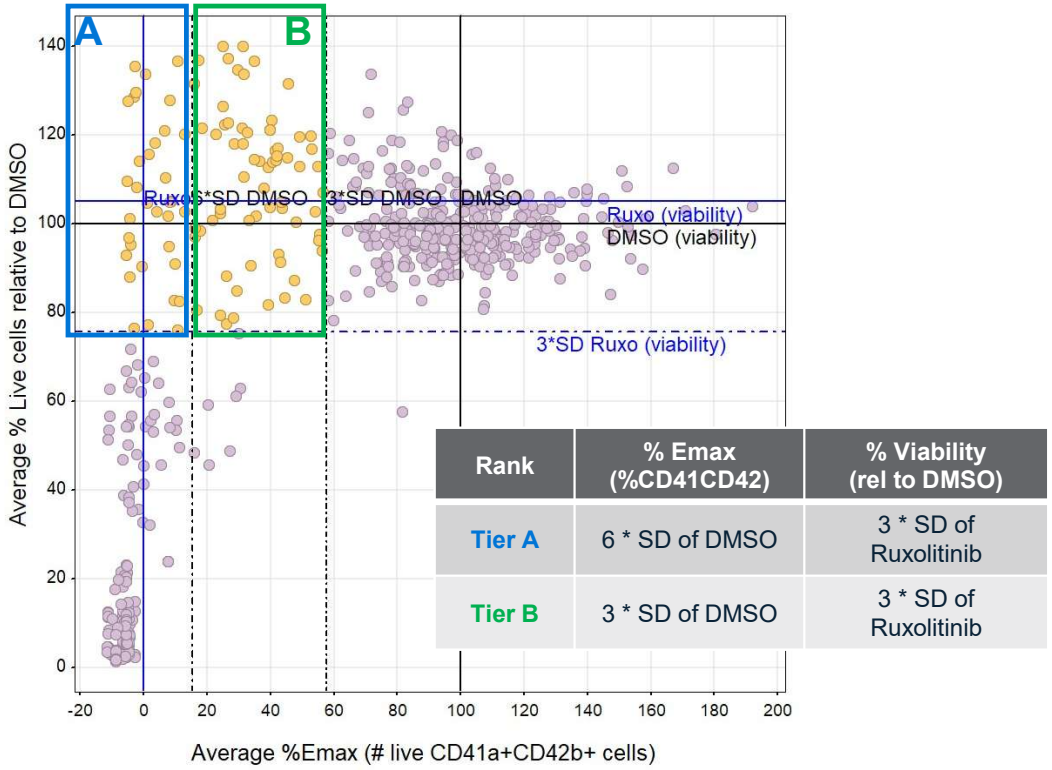




- JAK2 ^{V617F} iHSCs captured the cell states of interest and the clinical cell behavior (gene signature)
- JAK2 ^{V617F} iHSC cultured in the absence of TPO resulted in cytokine independent megakaryopoeisis and were responsive to ruxolitinib

ML guided predictions identified molecules that decreased TPO independent megakaryopoiesis

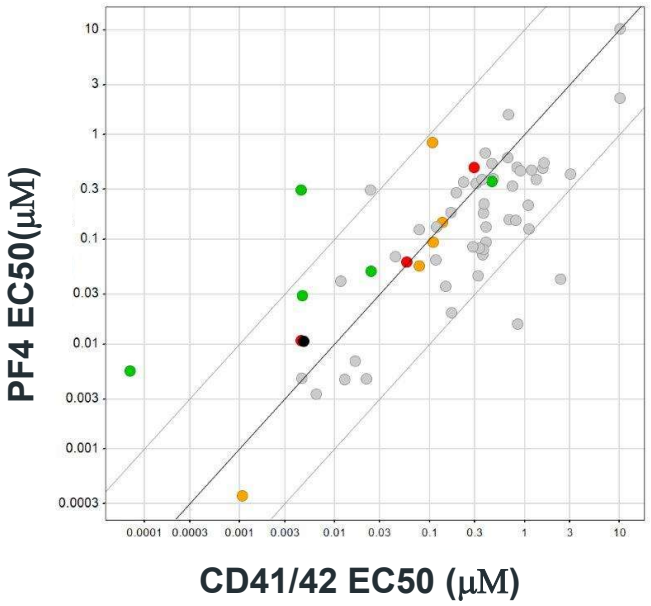
- Using our JAK2^{V617F} iHSC we tested our predictions at single dose using our CD41/CD42 flow cytometry assay
- Molecules were tiered based on their ability to inhibit TPO independent megakaryopoiesis while maintaining cell viability
- 18% of tested predictions were phenotypic hits and moved to dose response assessment.



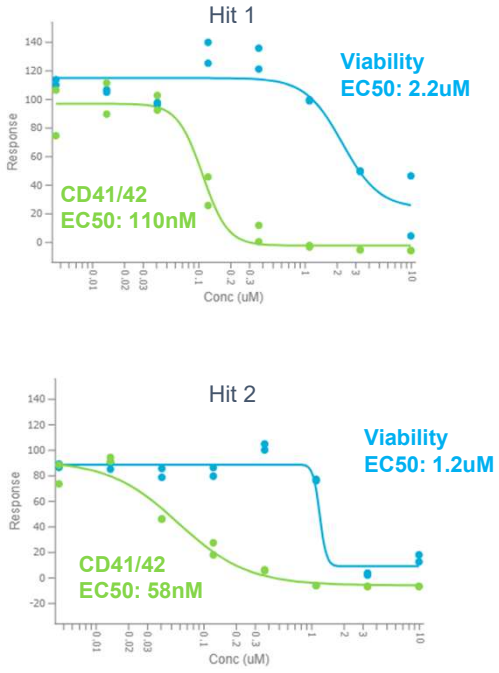
Rank	% Emax (%CD41CD42)	% Viability (rel to DMSO)
Tier A	6 * SD of DMSO	3 * SD of Ruxolitinib
Tier B	3 * SD of DMSO	3 * SD of Ruxolitinib

Multiple hits were identified and validated in primary MF patients CD34+ cells

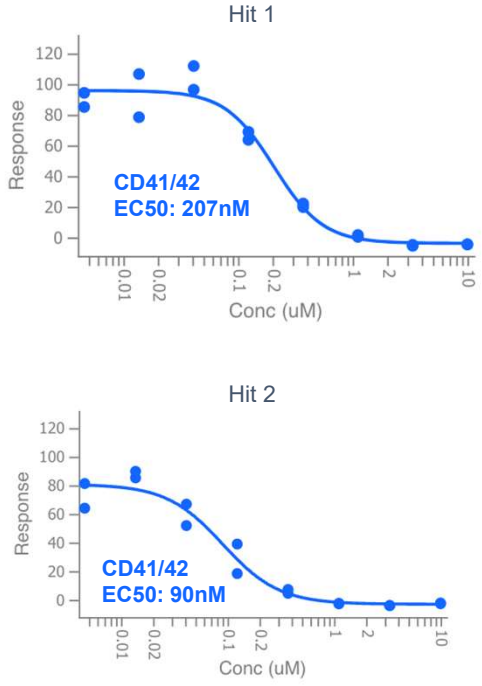
Novel, emerging and clinical targets identified



iPSC derived JAK2-KI iHSC

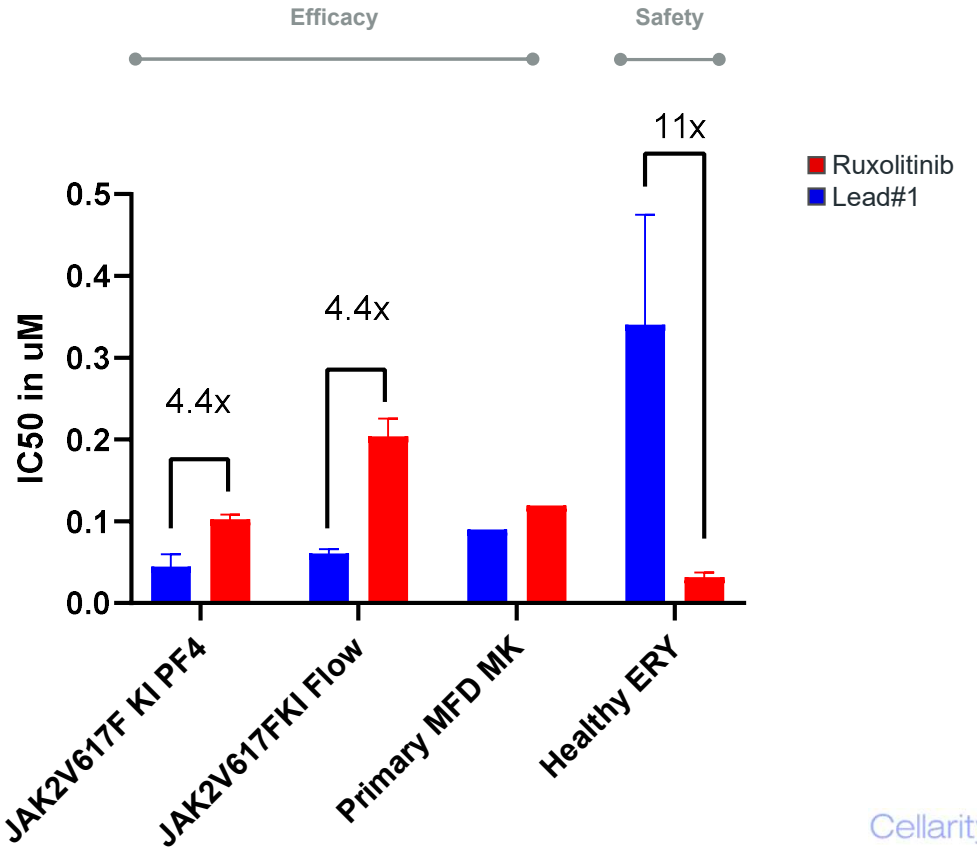


MF patients primary CD34+



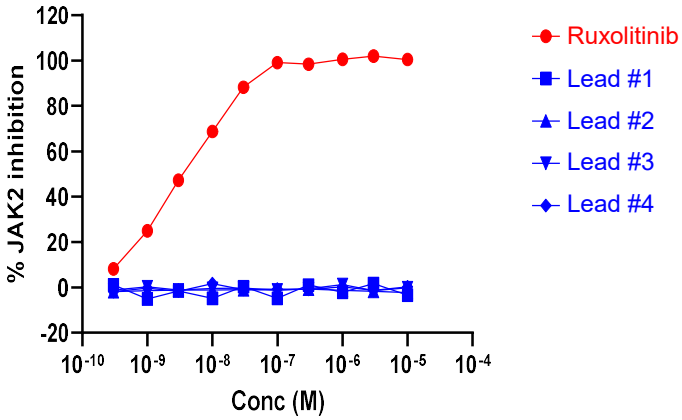
A counter-screen was used to identify lead molecules that spare healthy erythropoiesis in primary CD34+ cells

- Cytopenias (anemia, thrombocytopenia) are a common side-effect of JAK2 inhibitors due to importance of JAK signaling in hematopoiesis
- We established a counter-screen measuring the effect of our compounds in *in vitro* erythropoiesis using primary CD34+ cells
- Our leads demonstrate comparable efficacy to Ruxolitinib, while sparring healthy erythropoiesis



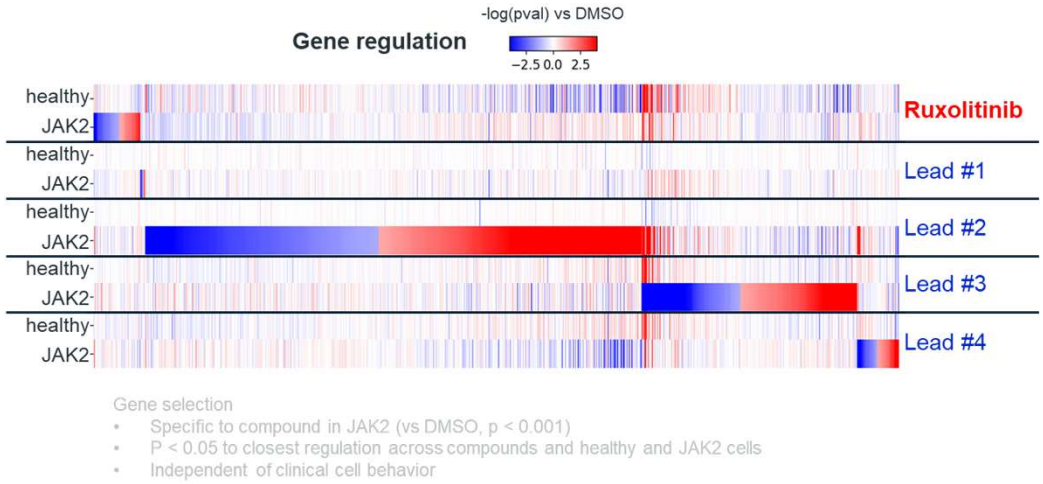
We have identified multiple MOAs differentiated from JAKi SOC, which would not be possible in a conventional target-based discovery

Biochemical JAK2 Kinase Assay



Our lead molecules are not JAK2 inhibitors

Disease-specific fingerprints can be linked to each lead compound



Each lead induces a different transcriptional signatures in JAK2 ^{V617F} HSPCs

Summary and Next Steps

- We built a **single cell atlas of myelofibrosis** to identify a cell-state transitions targeting the HSPC compartment
- We developed a JAK2^{V617F} iHSC *in vitro* model with **high translation** capturing the disease features of our clinical atlas and functional endpoints associated with myelofibrosis
- Leveraging our **ML guided discovery** engine, we identified small molecules that efficiently inhibit cytokine independent megakaryopoiesis
- Our lead molecules **are not** JAK2 inhibitors, and spare healthy erythropoiesis **differentiating** from approved JAK2 inhibitors

Next Steps

- Clonogenic potential of our lead molecules in healthy vs mutant HSPCs (**Q1 2026**)
- Lead molecules *in vivo* efficacy will be evaluated in JAK2^{V617F} transplant model (**1H 2026**)

Acknowledgments

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Golam Mohi

Bridget Marcellino

***Oral Presentation-Session 113**
Monday Dec 8th 3:15
Cly-124, a first-in-class DCN1 inhibitor partially suppresses CUL3 neddylation and induces fetal hemoglobin is a new potential treatment for sickle cell disease