# Differential MAPK Pathway Targeting for Improved Therapeutic Efficacy in Myeloproliferative Neoplasms

## Experimental Hematology / Oncology

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## INTRODUCTION

**Myeloproliferative neoplasms (MPN)** are hematopoietic malignancies with constitutive JAK2 signalling activation.

- **JAK2** activates the STAT3/5, PI3K/Akt and mitogen-activated protein kinase (MAPK) pathways.
- **Clinical JAK2 inhibitors (ruxolitinib)** control constitutional symptoms and splenomegaly.
- However, disease-modifiying effects are limited by **persistent MAPK activation**.
- JAK2 interacts with the MAPK pathway via **Src homology 2 domain-containing phosphatase 2** (**SHP2**), but the molecular link and optimal MAPK target remain unclear.

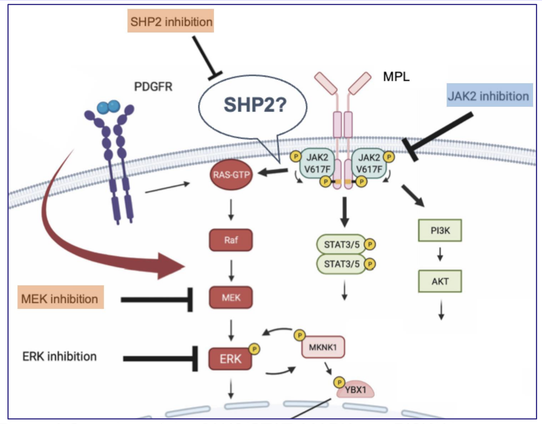


*Figure 1* Overview of the JAK2/STAT, MAPK and PI3K/Akt pathways.

## AIM & METHODS

We investigate the potential of **MAPK pathway targeting** at the level of SHP2 or MEK1/2 when combined with ruxolitinib and whether targeting both levels enhances efficacy.

1. **Genetic targeting** by shRNA-mediated knockdown of **SHP2**, **MEK** and dual **SHP2/MEK** in Ba/F3 cells stably expressing JAK2V617F or JAK2WT (wildtype) was evaluated.
2. **Pharmacological SHP2 and MEK inhibition** (by TNO155 and trametinib) was assessed and combined with ruxolitinib.
3. **As translational approach**, a Jak2V617F mutant mouse model was evaluated for corrective effects of dual **SHP2/MEK inhibition** combined with **JAK2 inhibition**.

## REFERENCES

[1]Stivala et al., JCI 2019
[2]Brkic et al., Leukemia 2021

## CONTACT INFORMATION

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## RESULTS

### *Genetic SHP2 and MEK inhibition sensitizes BaF3 mutant cells to JAK2 inhibition.*

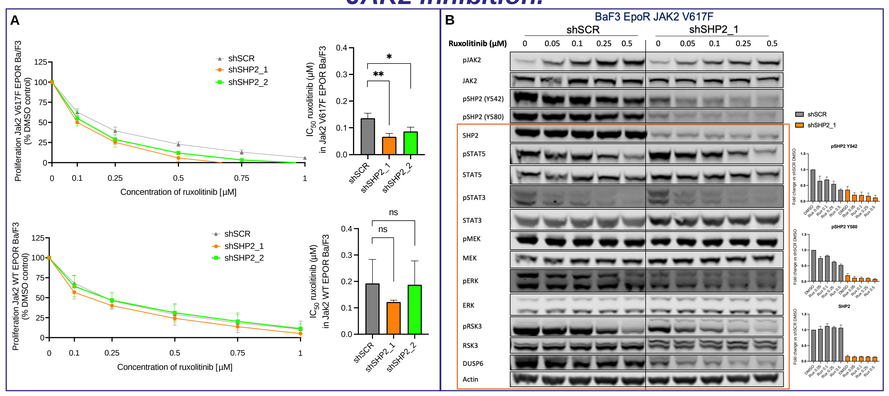


*Figure 2* SHP2 depletion sensitized BaF3 EpoR JAK2 V617F cells to ruxolitinib by lowering IC50 (**A**) and enhancing MAPK inhibition without affecting the JAK/STAT pathway (**B**).

### *Pharmacological JAK2/SHP2 and JAK2/MEK inhibition increases therapeutic efficacy in mutant cells.*

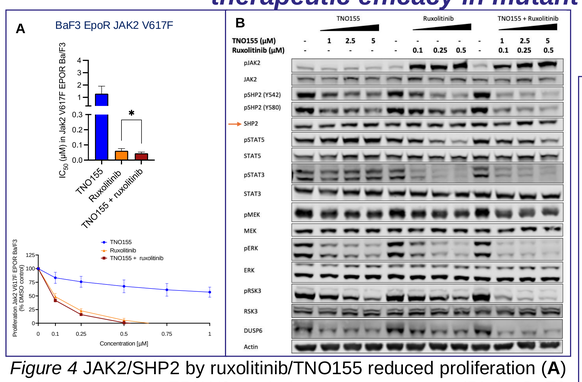


*Figure 4* JAK2/SHP2 by ruxolitinib/TNO155 reduced proliferation (**A**) and suppressed pERK1/2 and downstream targets (**B**) in BaF3 EpoR JAK2 V617F mutant cells.

### *In vivo: Combined SHP2/MEK inhibition with ruxolitinib reduces MPN progenitors.*

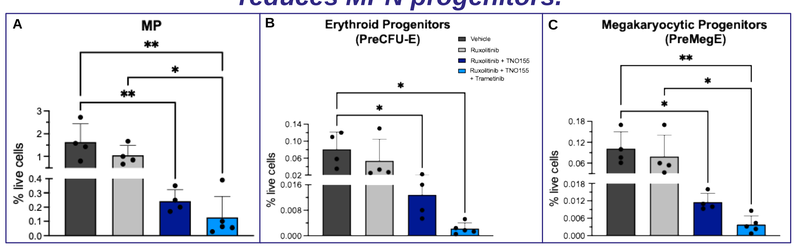


*Figure 7* Dual SHP2/MEK inhibition with ruxolitinib corrected MPN phenotype, reducing myeloid (MP) (**A**), erythroid (PreCFU-E) (**B**) and megakaryocytic (PreMegE) (**C**) progenitor cells.

### *Combined JAK2/SHP2/MEK pharmacological inhibition reduced mutant cell proliferation.*

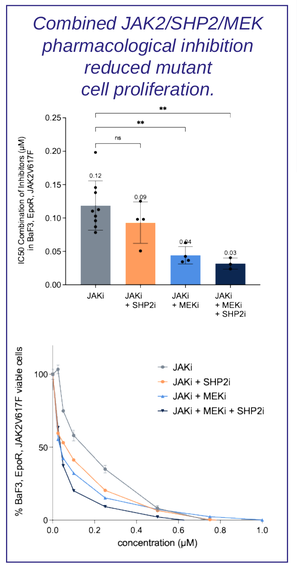


*Figure 6* Triple inhibition of JAK2, SHP2, and MEK by ruxolitinib, TNO155, and trametinib, respectively, decreased cell proliferation in mutant cells.

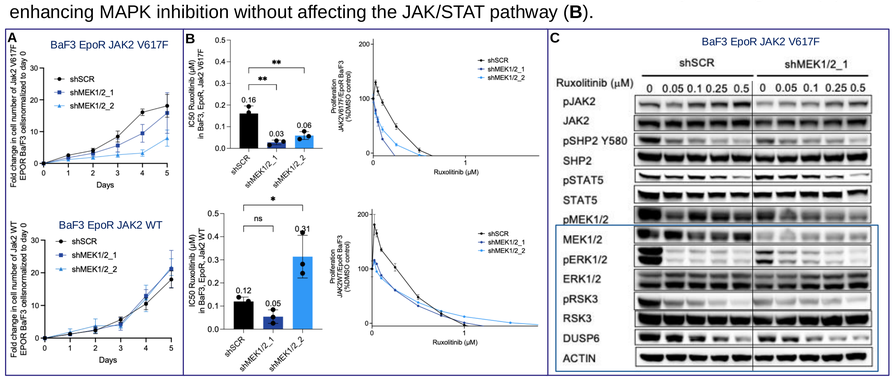


*Figure 3* MEK knockdown impaired mutant MPN cell growth (**A**), sensitized cell proliferation to the JAK2 inhibitor ruxolitinib (**B**), and concentration-dependently suppressed MEK-ERK downstream targets (**C**).

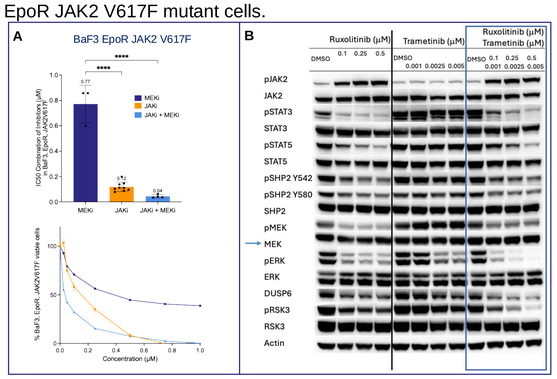


*Figure 5* JAK2/MEK by ruxolitinib/trametinib reduced proliferation (**A**) and suppressed pERK1/2 and downstream targets (**B**) in BaF3 EpoR JAK2 V617F mutant cells.

## CONCLUSION

Targeting the MAPK pathway at one or multiple levels **enhances therapeutic efficacy in MPN**, **increasing ruxolitinib sensitivity** by reducing cell proliferation and suppressing MAPK targets. Our findings highlight the therapeutic benefits of inhibiting **JAK2, SHP2, and MAPK pathway** intersections.

## OUTLOOK

→ Further **combined MAPK/SHP2 genetic inhibition** studies will deepen our mechanistic understanding and clarify the potential of this approach to enhance therapeutic outcomes.

→ **Functional MAPK pathway screening** (by using e.g., phosphoproteomics analyses, RNA sequencing) will elucidate the wiring of this pathway on a mechanistic level.

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