The conformation-selective PI3Kδ inhibitor roginolisib synergizes with JAK inhibitors in progenitor cells from naïve and JAK inhibitor-refractory/resistant patients with myelofibrosis

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INTRODUCTION

Myelofibrosis (MF) is driven by constitutive JAK2/STAT signaling associated with JAK2, CALR, and MPL mutations, leading to the development of JAK inhibitors (JAKi). However, additional pathways, including PI3K/AKT/mTOR, contribute to disease biology and JAKi resistance(1). Pan-PI3K inhibitors have shown activity but were limited by toxicity and isoform-selective PI3K inhibitors are now being developed that may have less toxicity. Roginolisib (IOA-244) is a conformation-selective, non-ATP-competitive, PI3Kδ inhibitor currently undergoing clinical trials in solid and hematologic malignancies(2).

AIM

To evaluate the activity of roginolisib alone and in combination with JAK inhibitors in MF models and primary progenitor cells.

METHOD

Using the JAK2V617F-mutated UKE-1 cell line and primary MF patient-derived CD34+ cells, mononuclear cells, and T lymphocytes, we evaluated the effects of treatment on proliferation, clonogenic potential, cellular interactions in co-culture, and cytokine production. Numerical data reported throughout this work are presented as the mean ± standard deviation (SD) of experiments carried out in triplicate.
The Student's t-test was employed to assess statistical significance, and the difference between values was considered significant at $P \leq 0.05$.

CONCLUSIONS

Roginolisib shows activity in primary MF cells and demonstrates strong synergy with JAK inhibitors. These results support the ongoing Phase Ib trial of roginolisib in MF patients with sub-optimal response to JAKi (HEMAMED, NCT06887803).

RESULTS

1) Roginolisib inhibits PI3K signaling pathway in MF cell lines

In UKE-1 cells, IOA-244 reduced phosphorylation of AKT, mTOR, and p70S6K (Fig. 1A), causing decreased cell growth (Fig. 1B).

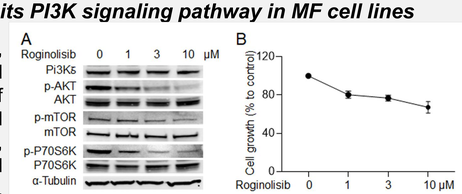


Figure 1. The effects of roginolisib on the UKE-1 cell line.

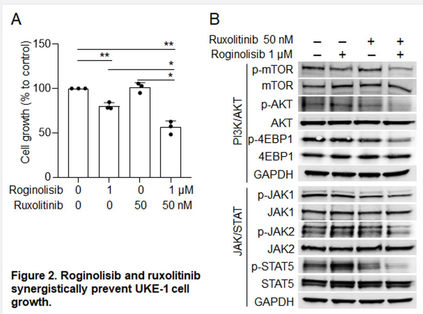
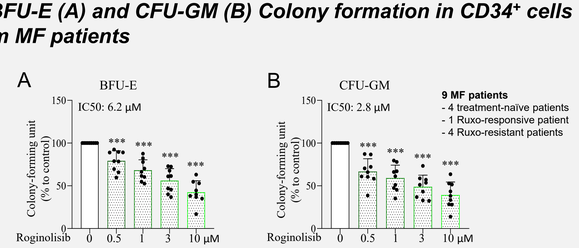


Figure 2. Roginolisib and ruxolitinib synergistically prevent UKE-1 cell growth.

Combination of rogi and ruxo synergistically significantly decreased cell growth versus control and single-agent (Fig. 2A; CI<1). Western blot showed dephosphorylation of main AKT/mTOR and JAK/STAT components (Fig. 2B) by drug combination.

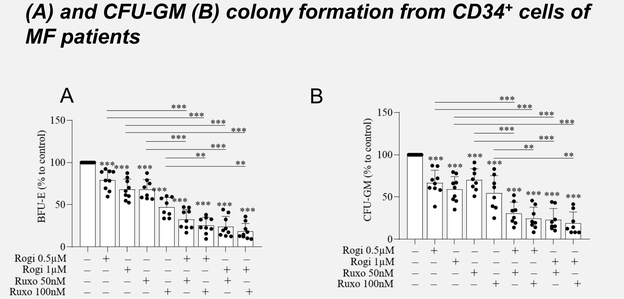
2) Roginolisib monotherapy induces dose-dependent inhibition of BFU-E (A) and CFU-GM (B) Colony formation in CD34⁺ cells from MF patients



Comparative analysis revealed that the treatment-naïve subset exhibited significantly greater sensitivity than Ruxolitinib-refractory (R/R) subset.

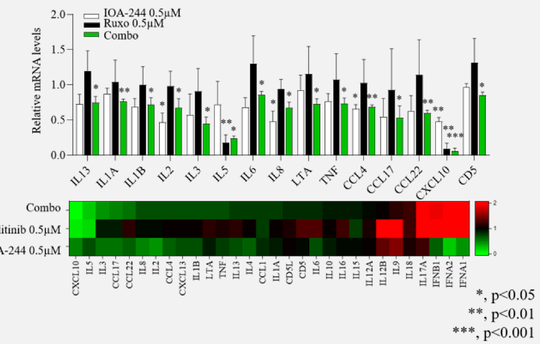
Roginolisib		%inh. @1 μM	%inh. @3 μM	%inh. @10 μM	IC50 (μM)
BFU-E	All pts	31.9±4.1	43.9±4.7	57.5±4.5	6.18
	Naive pts	37.7±4.9	53.9±3.9	66.6±3.9	3±0.9
	R/R pts	24.6±4.7	31.4±2.7	46.2±3.8	>10
CFU-GM	All pts	41±5.1	51.2±4.5	60.8±5.1	2.8
	Naive pts	51.9±3.9	60.7±3.3	70.6±4.4	1.4±0.6
	R/R pts	27.3±3.5	39.3±4.4	48.5±5.2	>10

3) Roginolisib and ruxolitinib synergistically inhibit BFU-E (A) and CFU-GM (B) colony formation from CD34⁺ cells of MF patients



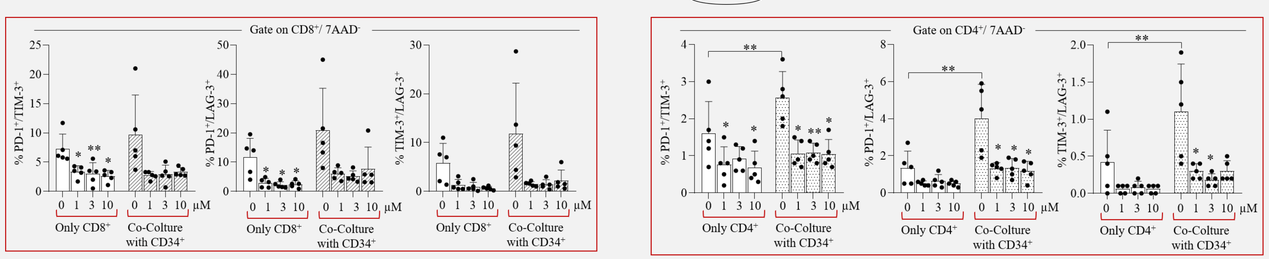
Roginolisib/Ruxolitinib		%inh. @0.5 μM	%inh. @0.05 μM	%inh. combo
BFU-E	All pts	20.8±4	31.6±3.9	67.2±3.9
	Naive pts	25.4±6.1	41.5±0.9	69.7±5.9
	R/R pts	15.2±3.6	21.8±3.6	64.1±5.2
CFU-GM	All pts	33.3±5	29.7±4.4	69.2±4.4
	Naive pts	43.2±4.7	37.2±3.9	74.6±4
	R/R pts	20.9±4.5	22.3±6.8	60.4±8

4) Roginolisib downregulates the expression of proinflammatory markers, alone and in combination with ruxolitinib



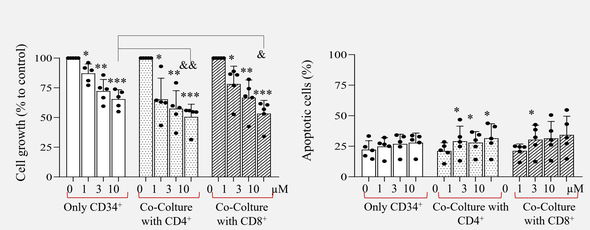
Roginolisib in monotherapy and combination with ruxolinitib downregulated the mRNA levels of inflammatory cytokines. Heatmaps show all cytokines tested, while bar graphs show all significant changes. Data are represented as fold changes compared to baseline.

5) Roginolisib decreases T cell exhaustion in ex-vivo CD34+/T cell co-cultures



CD34⁺ cells and T cells isolated from spleen of 5 MF patients were used to establish a co-culture system. The two populations were separated by a 3 μm transwell indicated for cell-cell interaction experiments. After 4 days, CD3⁺CD8⁺ or CD3⁺CD4⁺ were analysed by flow-cytometry for the checkpoints PD-1⁺ and TIM-3⁺ and LAG-3⁺

6) Roginolisib suppresses CD34⁺ cells and promotes apoptosis through direct and immune-mediated mechanisms



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CONTACT INFORMATION

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