THE SELECTIVE PI3Kδ INHIBITOR ROGINOLISIB SYNERGIZES WITH RUXOLITINIB AGAINST PROGENITOR CELLS FROM NAÏVE AND JAKi-REFRACTORY/RESISTANT PATIENTS WITH MYELOFIBROSIS

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INTRODUCTION

- Given the driver mutation-associated dysregulation of the JAK/STAT pathway, treatment of myelofibrosis (MF) has seen a fundamental shift with the introduction of JAK inhibitors.
- Beyond JAK/STAT, other key signaling pathways, such as the PI3K/AKT/mTOR, are also implicated in regulating cell growth, survival, proliferation, and metabolism in MF.
- Addressing the toxicity concerns of earlier PI3K inhibitors, isoform-selective PI3K inhibitors are now being developed (1,2).

AIM

This study aims to evaluate the efficacy of the first-in-class non-ATP-competitive PI3Kδ inhibitor, roginolisib (Rogi; IOA−244), as a single agent and in combination with Ruxolitinib (Ruxo), in *JAK2*V617F mutated cell lines and in-vitro models of MF primary progenitor cells.

METHOD

We used JAK2V617F-mutated UKE-1 cell line and primary cells (CD34⁺, mononuclear and T-lymphocytes from MF patients.

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≤0.05.

RESULTS

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

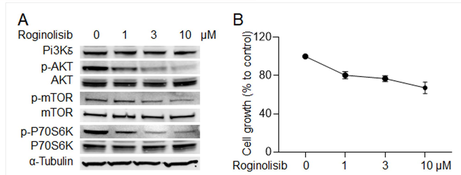

Figure 1. The effects of roginolisib on the UKE-1 cell line.

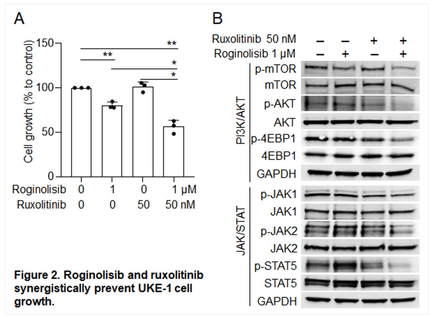

Figure 2. Roginolisib and ruxolitinib synergistically prevent UKE-1 cell growth.

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.

Roginolisib significantly reduced BFU-E and CFU-GM colonies (Table 1). Comparative analysis revealed that the treatment-naïve subset exhibited significantly greater sensitivity than Ruxolitinib-refractory (R/R) subset.

Roginolisib		1 μM	3 μM	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

Table 1. Roginolisib's effects on CD34⁺ cell clonogenic assays from 9 MF patients (5 treatment-naïve, 4 R/R).

Combining rogi with ruxo significantly reduced colony formation and exhibited synergism (CI<1). A comparative analysis showed that R/R patients responded similarly to the treatment-naïve group

Roginolisib/Ruxolitinib		0.5 μM	0.05 μM	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

Table 2. The effects of combining roginolisib with ruxolitinib on CD34⁺ cell clonogenic assays from 9 MF patients (5 treatment-naïve, 4 R/R).

Combining roginolisib with momelotinib significantly reduced colony formation and exhibited synergism (CI<1).

Roginolisib/ Momelotinib	0.5 μM	5 nM	Combo
BFU-E	11±2.1	19.4±0.9	64.2±0.6
CFU-GM	34.1±0.4	23±0.5	72.6±1.2

Table 3. The effects of combining roginolisib with momelotinib on CD34⁺ cell clonogenic assays from MF patients.

Mononuclear cells from 3 MF patients were treated with increasing doses of rogi for 3hr. Real-time PCR showed significant decrease in the mRNA of 10/30 cytokines (Fig. 3).

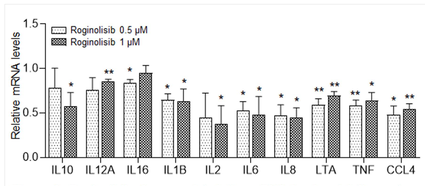

Figure 3. Roginolisib downregulated the mRNA levels of inflammatory cytokines

Splenic CD34⁺ and T cells from 5 MF pts were exposed (monoculture and CD4⁺/ CD8⁺ co-culture) to rogi doses for 4 days.
Roginolisib induced dose-dependent CD34⁺ cell growth inhibition (Fig. 4A) that was associated with increased apoptosis (Fig. 4B).

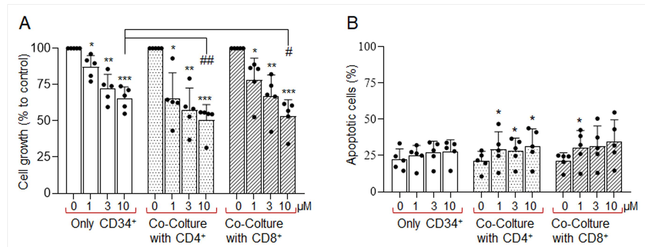

Figure 4. Cell growth and viability in CD4⁺CD34⁺ and CD8⁺CD34⁺ co-cultures under roginolisib

Flow cytometry was used to assess checkpoint marker expression on CD8⁺, CD4⁺ and Treg cells. Across all T cell subsets, co-culture increased membrane TIM-3 and LAG-3 expression compared to monoculture, an effect which was significantly reduced by roginolisib treatment (Fig. 5).

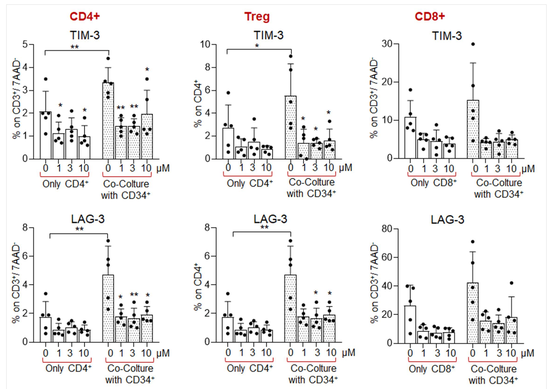

Figure 5. Roginolisib downregulated membrane expression of LAG-3 and TIM-3 on CD4⁺, CD8⁺, and Treg cells in CD34⁺/CD4⁺ and CD34⁺/CD8⁺ co-culture systems.

CONCLUSIONS

These findings support efficacy of roginolisib, a selective PI3Kδ inhibitor, in primary MF cells, especially when combined with JAK2 inhibitors, providing a rationale for the ongoing phase 1/2 HEMA-MED clinical trial (NCT06887803), currently ongoing in Italy and Spain.

REFERENCES

1. **Johnson Z. et al.** OA-244 is a Non–ATP-competitive, highly selective, tolerable PI3K Delta inhibitor that targets solid tumors and breaks immune tolerance. Cancer research Communication 2023; 3(4):576-591.

2. **Siempelkamp B.D. et al.** Molecular mechanism of activation of class IA phosphoinositide 3-kinases (PI3Ks) by membrane-localized Hras JBC 2017; 21;292(29):12256-12266.

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