Novel Pi3kδ Inhibitor Roginolisib Synergizes with the Bcl-2 Inhibitor Venetoclax in Hematological Malignancies

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INTRODUCTION

- Roginolisib (IOA-244) is a first in class allosteric modulator and non-ATP competitive, selective PI3Kδ inhibitor currently in a Phase 1 clinical study in lymphoma and solid tumors.
- Initial structural and biochemical studies identified unique chemical structure, excellent selectivity, and excellent PK properties for roginolisib as compared to earlier PI3Kδ inhibitors, while the clinical trial has demonstrated a favorable safety profile[1].
- Consistent with prior PI3Kδ inhibitors, roginolisib inhibits the in vitro growth of lymphoma cells.
- In contrast to other PI3Kδ inhibitors, roginolisib activity is correlated with the expression levels of PIK3CD, suggesting specific and on target cancer cell-intrinsic effects[2].

AIM

- Identify best combination partners for roginolisib in hematological malignancies
- Investigate the potential synergy of roginolisib with venetoclax in CLL patients treated with BTKi

METHODS

CLL samples: Blood samples collected from patients treated with BTKi for at least 6 months were banked according to our IRB approved tissue banking protocol. Informed consent was obtained from all patients. Frozen PBMCs were used for the study.

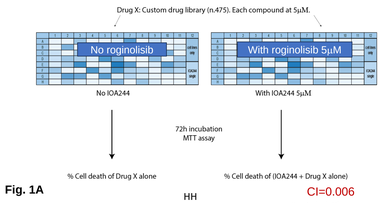
MTT Assay: The antiproliferative effect of single and combination treatments in hematologic cancer cell lines was determined by adding MTT reagent to each well and the plates were incubated at 37°C for 4 hours, followed by addition of sodium dodecyl sulfate lysis buffer. The lysed cells were kept overnight, and then the absorbance was read. The effect of the combinations was determined according to the Chou-Talalay Combination Index (CI) calculated with the Synergy R package.

Apoptosis Assay: CLL cells were allowed to acclimatize in co-culture with HS-5 stromal cells for 24h prior to the addition of drugs. The cells were collected 48 hours after drug treatment. CLL cells were gated as CD5 and CD19 double positive and the viable cells were double negative for annexin and 7AAD.

RESULTS

A pharmacological screen identified synergy of Roginolisib with Bcl2 inhibitors in haematological malignancies

A screen to identify active roginolisib-based combinations was done in 2 cell lines, SP-53 (mantle cell lymphoma, MCL), and HH (cutaneous T cell lymphoma, CTCL)



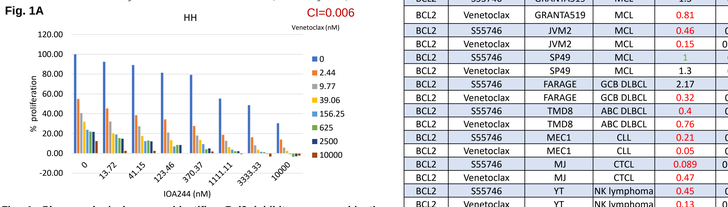


Fig. 1. Pharmacological screen identifies Bcl2 inhibitors as combination partners. MTT assay showed synergy in cutaneous T cell lymphoma cell line HH (**1A**) and mantle cell lymphoma cell line SP53 (**1B**). Calculation of Combination Index (CI) was done by Chou-Talalay method. Combination Index: CI<0.3, strong synergism; 0.3-0.9, synergism; 0.9-1.1 additive effect.

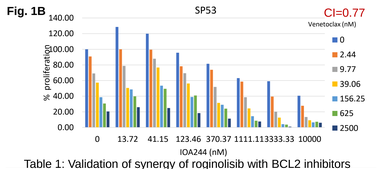


Table 1: Validation of synergy of roginolisib with BCL2 inhibitors

Target	Combination partner	Cell line	Lymphoma subtype	Median Combination Index	95% C.I.
BCL2	S55746	GRANTA519	MCL	1.3	0.56-1.5
BCL2	Venetoclax	GRANTA519	MCL	0.81	0.66-1
BCL2	S55746	JVM2	MCL	0.46	0.18-0.75
BCL2	Venetoclax	JVM2	MCL	0.15	0.096-0.21
BCL2	S55746	SP49	MCL	1	0.56-1.5
BCL2	Venetoclax	SP49	MCL	1.3	0.44-1.9
BCL2	S55746	FARAGE	GCB DLBCL	2.17	1-3.1
BCL2	Venetoclax	FARAGE	GCB DLBCL	0.32	0.24-0.56
BCL2	S55746	TMD8	ABC DLBCL	0.4	0.35-0.51
BCL2	Venetoclax	TMD8	ABC DLBCL	0.76	0.5-5
BCL2	S55746	MEC1	CLL	0.21	0.15-0.31
BCL2	Venetoclax	MEC1	CLL	0.05	0.03-0.08
BCL2	S55746	MJ	CTCL	0.089	0.035-0.53
BCL2	Venetoclax	MJ	CTCL	0.47	0.3-0.8
BCL2	S55746	YT	NK lymphoma	0.45	0.17-0.63
BCL2	Venetoclax	YT	NK lymphoma	0.13	0.062-0.49

MTT assay was carried out using combination of roginolisib with Bcl2 inhibitors venetoclax or S55746 in various lymphoma cell line. MCL (Mantle Cell Lymphoma), DLBCL (Diffuse Large B Cell Lymphoma), CLL (Chronic Lymphocytic Leukemia), CTCL (Cutaneous T Cell Lymphoma). Combination Index: CI<0.3, strong synergism; 0.3-0.9, synergism; 0.9-1.1 additive effect.

Roginolisib shows comparable tumor intrinsic and cell killing abilities in CLL patient samples responding or progressed on BTKi

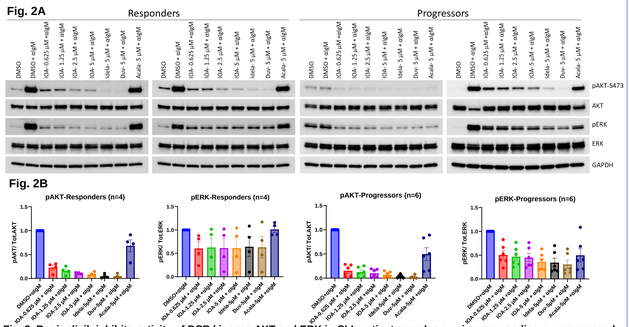


Fig. 2. Roginolisib inhibits activity of BCR kinases AKT and ERK in CLL patient samples ex vivo, responding or progressed on BTKi. Changes in BCR kinase activity of AKT and ERK proteins induced by treatment with roginolisib in CLL cells. Levels of pAKT, pERK, AKT, ERK and GAPDH protein were analyzed by immunoblotting in patient samples responded (n=4) or progressed (n=6). Representative immunoblot images are shown from two samples in each group (**2A**). Densitometry quantification of immunoblots were done using imageJ and shown (**2B**).

Fig. 3

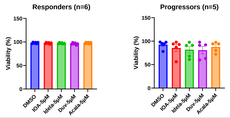


Fig. 3. Roginolisib showed comparable cell killing activity to other BCRi such as idelalisib, duvelisib or acalabrutinib. Viability of CLL cells following 48h of ex vivo treatment with the drugs as indicated. Percentage of viable cells were determined by Annexin/7AAD staining.

Roginolisib and Venetoclax show high synergy in killing CLL patient-derived tumor cells

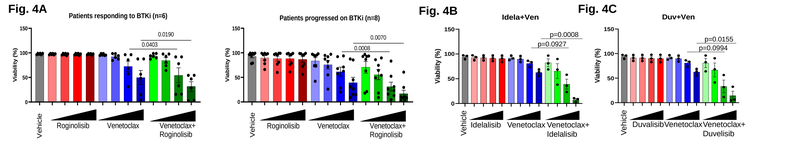


Fig. 4: Roginolisib + venetoclax display synergistic activity in CLL patient samples. Viability of CLL cells following 48h of ex vivo treatment with increasing concentrations of roginolisib (0.625, 1.25, 2.5 or 5 μM) and venetoclax (1, 2, 4 or 8 nM) alone or in combination as indicated in the figure in samples responding to BTKi and samples progressed on BTKi (**4A**). Synergy observed with roginolisib was comparable to the synergy observed with idelalisib (**4B**) or duvelisib (**4C**) + venetoclax combination. The percentage of viable cells were determined by Annexin/7AAD staining. Statistical analysis was done using paired t-tests.

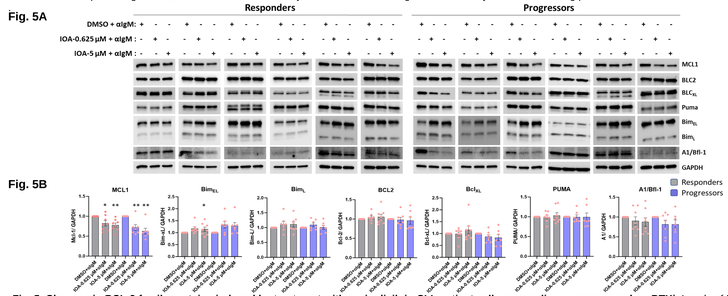


Fig. 5. Changes in BCL-2 family proteins induced by treatment with roginolisib in CLL patient cells responding or progressed on BTKi. Levels of MCL1, BIM (BIM_{EL} + BIM_L), BCL2, BCL_{XL}, A1/Bfl-1 and GAPDH protein were analyzed following by immunoblotting in patient samples responded (n=8) or progressed (n=7). Representative immunoblot images are shown from two samples in each group (**5A**). Densitometry quantification of immunoblots were done using imageJ and shown (**5B**). Statistical analysis was done using paired t-test. Data shown are mean ± SEM of samples. *p < 0.05, **p < 0.01.

Roginolisib shows best in class clinical safety profile

Table 2: Clinical safety profile of roginolisib compared to other PI3K inhibitors

	Parameter	roginolisib	idelalisib	duvelisib	umbralisib	parsaclisib	zandelisib	copanlisib
Drug use	Discontinuation	0%	24%	35%	15%	16%	5%	23%
	Continuous dosing	yes	yes	yes	yes	no	no	no
	Combo potential	●	●	●	●	●	●	●
	CLL develop. status	ongoing	approved	approved	NA	NA	discont.	discont.
Safety profile	Tolerability (SAE≥G3)	●	●	●	●	●	●	●
	Diarrhea/colitis	0%	14%	23%	7%	9%	4%	5%
	Infection	0%	23%	27%	20%	3%	15%	23%
	Neutropenia	0%	17%	43%	11%	7%	14%	21%
	ALT/AST increase	0%	18%	8%	7%	1%	1%	2%
	PK	●	●	●	●	●	●	iv
	Metabolism	●	●	●	●	?	?	●
Chemistry	Chemotype	unique	idelalisib	idela-like	idela-like	idela-like	unique	unique
	Selectivity	●	●	●	●	●	●	●
	ATP competitive	no	yes	yes	yes	yes	yes	yes

● Optimal
● Suboptimal
● Unfavorable

CONCLUSIONS

- ❑ Initial screening identified that roginolisib synergized with the Bcl-2 inhibitor venetoclax in the HH and SP53 cell lines.
- ❑ Synergy was observed with both venetoclax and another Bcl2-inhibitor S55746 in various hematologic malignancies.
- ❑ Roginolisib showed comparable tumor intrinsic and cell killing abilities to other BCRi in CLL patient derived tumor cells.
- ❑ Roginolisib synergizes with venetoclax in CLL derived tumor samples whether responding to or progressed on BTKi.
- ❑ Mechanistically, roginolisib sensitizes CLL cells to venetoclax via modulating the Bcl2 family proteins MCL1 and BIM.
- ❑ The novel PI3Kδ selective inhibitor roginolisib shows promising preclinical activity. Roginolisib is the best-in-class PI3Kδ inhibitor, with an exceptional safety profile to date and our data support extending this combination to clinical trials in hematological malignancies.

REFERENCES

1. Di Giacomo. A. M, et al., J Clin Oncol 41, 2023 (suppl 16; abstr. 3110).
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