Patient derived tumor cells identify mechanistically rational combinations for the PI3Kdelta inhibitor roginolisib in solid and haematologic malignancies.

Chiara Riganti[1], Chiara Tarantelli[2], Elisa Civanelli[2], Binu Kandathilparambil Sasi[3], Michael Lahn[4], Lars van der Veen, Giusy Di Conza[4], Francesco Bertoni[2], Jennifer R Brown[3]

[1] Department of Oncology, University of Torino, via Santena 5/bis, 10126 Torino, Italy, [2] Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Bellinzona, Switzerland, [3] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA, [4] iOnctura SA, Avenue de Sécheron 15, 1202 Geneva, Switzerland

Summary

Introduction

Roginolisib (IOA-244) is a first in class allosteric modulator and non-ATP competitive PI3Kδ inhibitor currently in a Phase 1b clinical study. In previous preclinical studies, roginolisib inhibits suppressive immune cells, such as Tregs and myeloid-derived suppressive cells (MDSC), while preserving proliferation and function of CD8 T cells. 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.

Material and methods

We have used patient derived tumor cells to evaluate roginolisib in combination with immune- or tumor-targeted therapies to identify synergies that could be translated to future clinical studies.

Results

Here, we show that in two *ex vivo* co-culture models of patient-derived mesothelioma cells with matched PBMC, the addition of roginolisib to cisplatin plus nivolumab specifically increased activated Ki67+/IFNg+ CD8 T cells and M1-like macrophages, and concomitantly decreased exhausted TIM3+ CD8 T cells, Tregs, M2-like macrophages and MDSCs with an overall effect to increase the antitumoral immune response.

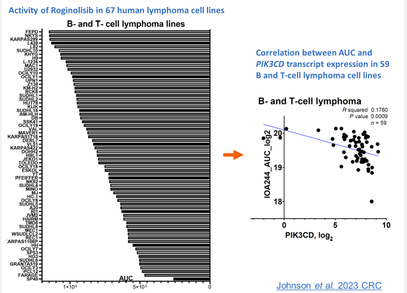
A screen of 474 compounds in combination with roginolisib in one T cell and one B cell lymphoma cell lines prioritised several clinical compounds to test in patient derived tumor cells. In particular, Roginolisib synergised with BCL2 inhibitors in the cell lines, with validation in patient-derived CLL cells from patients who had developed resistance to prior BTK inhibitor treatment.

Conclusion

Fresh patient derived tumor cells are a powerful in vitro approach to identify potential combination to evaluate in cancer patients. Our data supports combining roginolisib with checkpoint inhibitors, for example in lung cancers, and targeted molecular therapies such as BCL2 inhibitors in CLL. The mechanistic synergy of these combinations has potential to provide greater patient benefit compared to the use of these medicines as single agents.

Background

Intrinsic Role:
Acting on the malignant immune cell (Bcell/Tcell/Stem cell)



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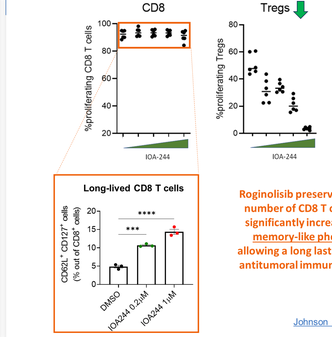
Antitumor activity of Roginolisib positively and significantly correlates with the expression levels of PI3Kδ

Extrinsic Role:
Preserving CD8 T cells, while decreasing Tregs



Roginolisib preserves the total number of CD8 T cells whilst significantly increasing their memory-like phenotype, allowing a long lasting, durable antitumoral immune response

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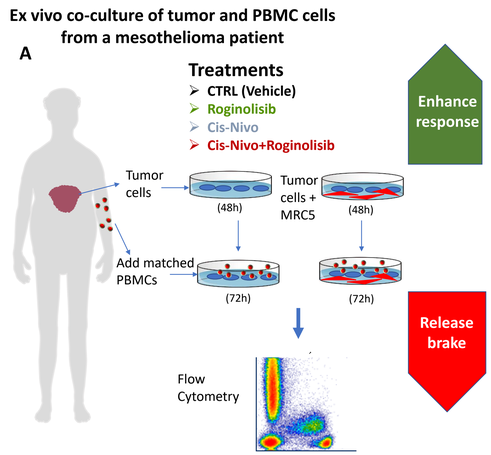
Roginolisib shows best in class clinical safety profile

	Parameter	Roginolisib^	Zydelig* (Gilead)	Copiktra* (Secura)	Ukoniq* (TG Ther.)	Parsaclisib (Incyte)	Zandelisib (MEI Phar.)
Drug use	Dose interruption AE related	no	41%	64%	45%	16%	5%
	Continuous dosing	yes	no	no	No	no	no
	Combination potential	●	●	●	●	●	●
	Lymphoma develop. status	active	withdrawn	withdrawn	withdrawn	withdrawn	withdrawn
	Solid tumor develop. status	active	halted	NA	NA	halted	NA
Safety profile	Tolerability (SAE ≥ Grade 3)	●	●	●	●	●	●
	Infection	0%	23%	27%	20%	0%#	0%#
	Neutropenia	2%$	28%	43%	17%	20%	16%
	Diarrhea or colitis	0%	14%	23%	7%	9%	5%
	ALT/AST increase	0%	18%	8%	7%	3%	8%

*FDA briefing document ODAC meeting April 21, 2022; ^ROGINOLISIB at RP2D of 80 mg QD #Mandatory pneumocystis jiroveci pneumonia (PJP) prophylactic treatment; $Transient decrease that resolved whilst continuing treatment

The safety profile of Roginolisib prompt us to design non-clinical research that would allow identification of combination therapies that could be explored in future clinical studies.

1. Roginolisib boosts the antitumoral immune response of immunotherapy and chemotherapy in solid tumors



- Data are biological duplicate and technical duplicates (2+2)
- Results confirmed in a second donor (BAP1 null)

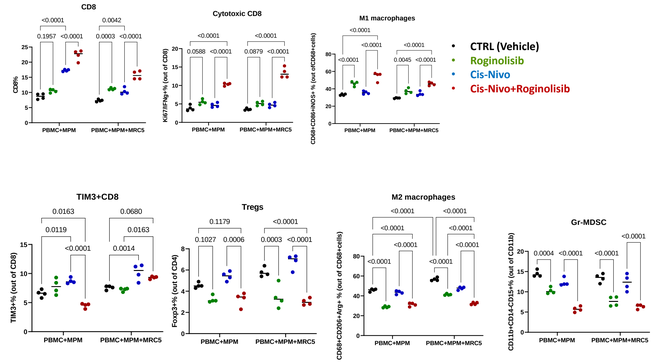


Fig. 1B Patient-derived BAP1-wild-type MPM cells were co-cultured with peripheral mononuclear cells (PBMCs) in the absence or presence of MRC5 fibroblasts and treated with roginolisib alone, co-incubated with cisplatin/nivolumab. The immunophenotype of immune cells was analyzed by flow cytometry (n = 2, two independent experiments). *p<0.05, **p<0.01, ***p<0.001.

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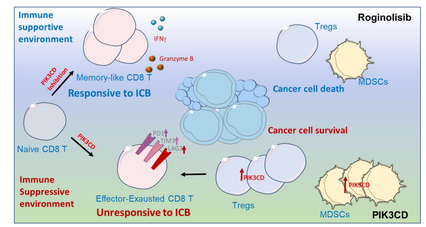

Bowers et al., Frontiers in Imm 2017
Wang et al., J. of Immunology 2022
Cannons et al., Cell reports 2021
Johnson et al., Cancer research comm 2023
Ali et al, Nature 2014

Fig. 1C Scheme describing the multipronged mechanism of action of Roginolisib in solid tumors: Roginolisib selectively decreases Tregs, while preserving CD8T cells. Within the CD8T cells, PI3Kd inhibition has been shown by us and others that increases the memory-like long-lived subsets. In addition to that, Roginolisib inhibits MDSCs.

2. A pharmacological screening identified synergy of roginolisib with Bcl2 inhibitors in haematological malignancies

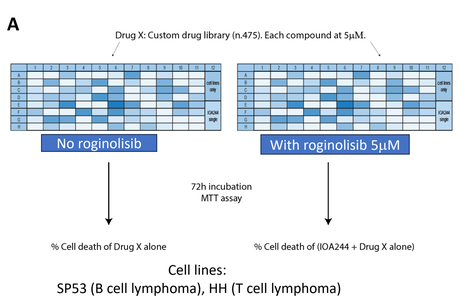

Fig. 2A A screen to identify active roginolisib-based combinations was done in 2 cell lines, SP-53 (mantle cell lymphoma, MCL), and HH (cutaneous T cell lymphoma, CTCL). Among others, combination partners

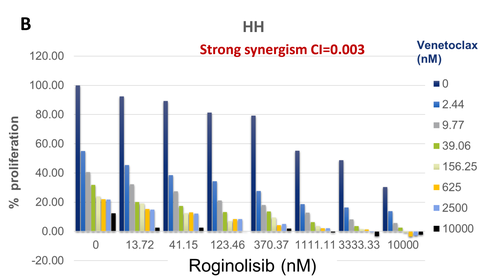

Fig. 2B Validations shown in HH cell lines performed with MTT assay after 72h treatment at the indicated doses
Calculation of Combination Index (CI) by Chou-Talalay Combination Index:
CI<0.3, strong synergism; 0.3-0.9, synergism; 0.9-1.1 additive effect

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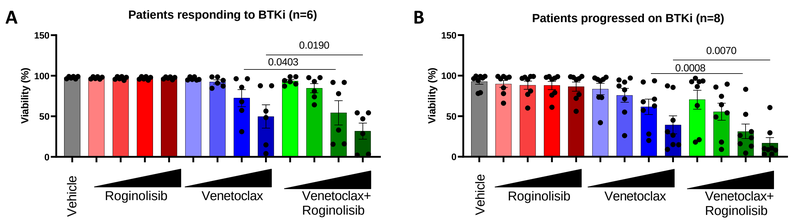
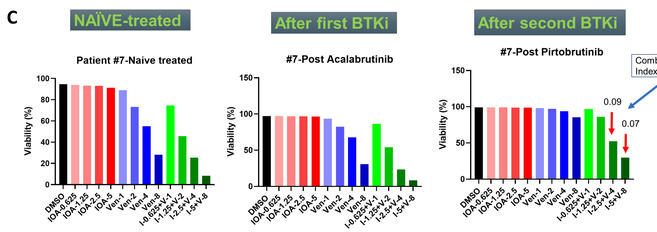
target	Combination partner	cell line	Lymphoma subtype	Median Combination Index	95% C.I.
BCL2	S55746	GRANTA519	MCL	1.3	0.56-1.5
BCL2	Venetoclax	GRANTA519	MCL	**0.81**	0.66-1
BCL2	S55746	JVM2	MCL	**0.46**	0.18-0.75
BCL2	Venetoclax	JVM2	MCL	**0.15**	0.096-0.21
BCL2	S55746	SP49	MCL	1	0.56-1.5
BCL2	Venetoclax	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

Fig. 2C. Table summarizing the validation of synergy of roginolisib with BCL2 inhibitors, in the indicated cell lines. Combination index was calculated, after performing proliferation assay.

3. Roginolisib and venetoclax show high synergy in reducing proliferation of BTK-resistant CLL patient-derived tumor cells



Viability of CLL following 48h of ex vivo treatment with increasing concentrations of roginolisib (0.625 to 5mM) and venetoclax alone (1 to 8nM) or in combination as indicated, in samples from patients responding to BTKi (**Fig. 3A**) or with progression on BTKi (**Fig. 3B**). Roginolisib also reverts resistance to venetoclax after prior treatment with two different BTK inhibitors (**Fig. 3C**). The percentage of viable cells was determined by Annexin/7AAD staining. Roginolisib and venetoclax were used at a constant ratio of 625:1. Statistical analysis used paired t-tests. Graphs show mean+/-sem. Each dot represents one patient.



Acalabrutinib and Pirtobrutinib are 2 BTKi approved by the FDA

Kapoor et al., 2020

Possible mechanism of action: inhibition of PI3Kδ by Roginolisib prevents compensatory upregulation of Mcl1 and overcome resistance to Bcl2 inhibitors

Contact: g.diconza@ionctura.com