The PI3Kδ inhibitor roginolisib (IOA-244) preserves T cell function and activity

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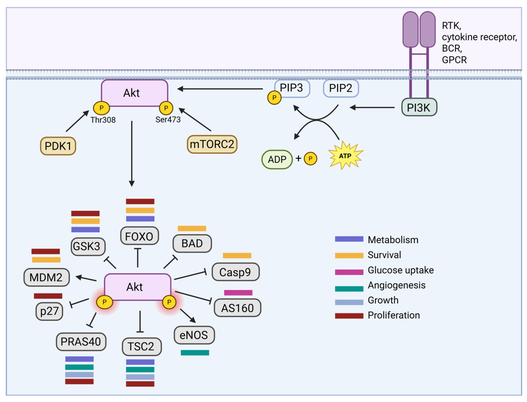
INTRODUCTION

The use of PI3K inhibitors have shown promise in some hematological cancers, but further label expansion of approved drugs and introduction of new candidates have been hampered by reports of serious immune-related adverse effects, particularly due to its effects on T cells. Thus, the development of novel and effective PI3K inhibitors that lack these adverse effects is highly desirable.



AIMS

We evaluated effects of the novel non-ATP competitive PI3Kδ inhibitor roginolisb (IOA-244) compared to the approved PI3Kδ inhibitor idelalisib, with a focus on its effects in human T cells. We did so by,

1. Validating function of roginolisib in a clinically relevant in vitro cancer model (chronic lymphocytic leukemia, CLL), and comparing these effects to those of the approved PI3K inhibitor idelalisib.
2. Evaluating effects of roginolisib and idelalisib on T effector cell signaling, proliferation and $CD8^+$ T cell cytotoxic abilities.
3. Evaluating effects of roginolisib and idelalisib on Treg activation and suppressive functions.
4. Evaluating effects of roginolisib and idelalisib on pro-inflammatory $CD4^+$ T cell helper subsets (Th1, Th2, Th17 and Tfh cells).

RESULTS

Idelalisib and roginolisib inhibit PI3K signaling and reduce viability of CLL B cells

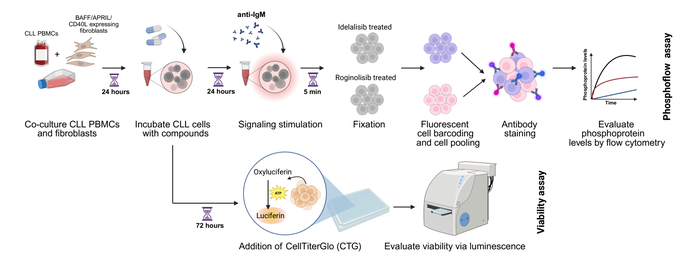


Figure 1: Experimental assays used to determine effects of roginolisib and idelalisib on PI3K signaling (phosphoflow) and CLL B cell viability (CellTiterGlo, CTG).

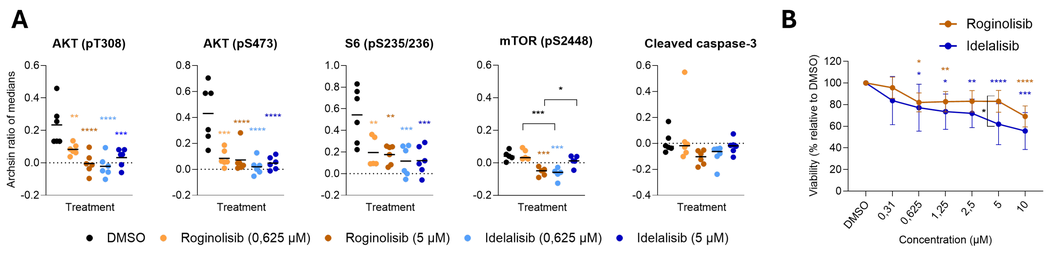


Figure 2: PBMCs were isolated from CLL patients, treated with DMSO or indicated concentrations of the drugs, and subjected to assays described in Figure 1**. A**) Roginolisib and idelalisib significantly inhibited phosphorylation of key signaling proteins downstream of PI3K in CLL B cells (n=3). **B)** Both drugs also inhibited CLL B cell viability in a concentration-dependent manner (n=3).

Idelalisib and roginolisib inhibit T cell function, idelalisib most potently inhibit T cell activation and proliferation

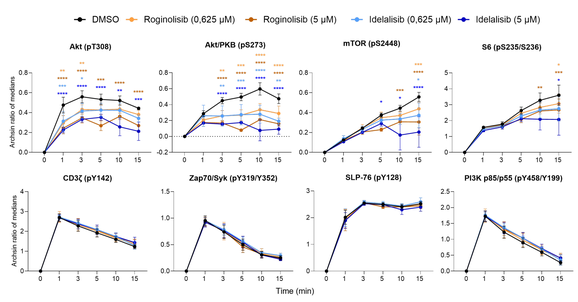


Figure 3: $CD3^+$ T cells isolated from healthy donors were treated with DMSO or indicated concentrations of roginolisib and idelalisib, before TCR signaling was initiated by addition of anti-CD3/2/28. The cells were fixed and stained with phospho-protein-specific antibodies and analyzed by flow cytometry. Roginolisib and idelalisib specifically and significantly inhibited PI3K-related signaling pathways in human T cells, whilst TCR-related signaling was left unaffected (n=3).

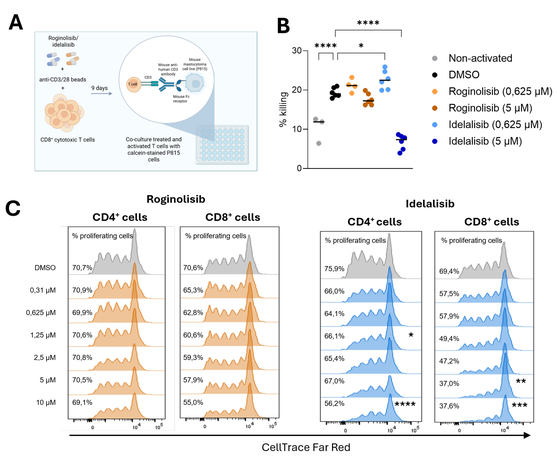


Figure 4: **A)** Schematic overview of cytotoxic killing assay. **B)** $CD8^+$ T cells were activated and treated with indicated concentration of the drugs. Roginolisib showed non-significant effects on T cell cytotoxic functions. 0,625 μM idelalisib caused a significant increase in cytotoxic capabilities, whilst 5 μM idelalisib caused a significant reduction in cytotoxic functions (n=5-6). **C)** $CD3^+$ T cells were activated and treated with indicated concentrations of the drugs, and proliferation was assessed by CellTrace staining and flow cytometry. $CD8^+$- nor $CD4^+$ T cell proliferation were significantly reduced by roginolisib, whilst idelalisib significantly reduced proliferation of $CD4^+$ T cells at 1,25 μM and 10 μM, and of $CD8^+$ T cells at 5 μ and 10 μM (representative image from n=3 donors).

Roginolisib and idelalisib modestly affect FoxP3 expression and Treg suppressive functions

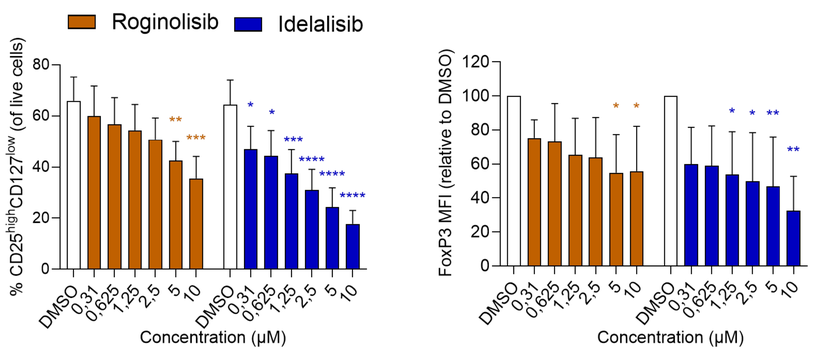


Figure 5: Tregs were isolated from healthy donors, stimulated with anti-CD3/2/28-coated beads and treated with DMSO or indicated concentrations of roginolisib or idelalisib . The cells were stained with relevant antibodies and analyzed by flow cytometry. Both roginolisib and idelalisib significantly reduced the percentage of $CD25^{high}CD127^{low}$ Tregs and FoxP3 MFI, but roginolisib only did so from 5 μM, whilst idelalisib already from 0,31 μM and 1,25 μM (n=3).

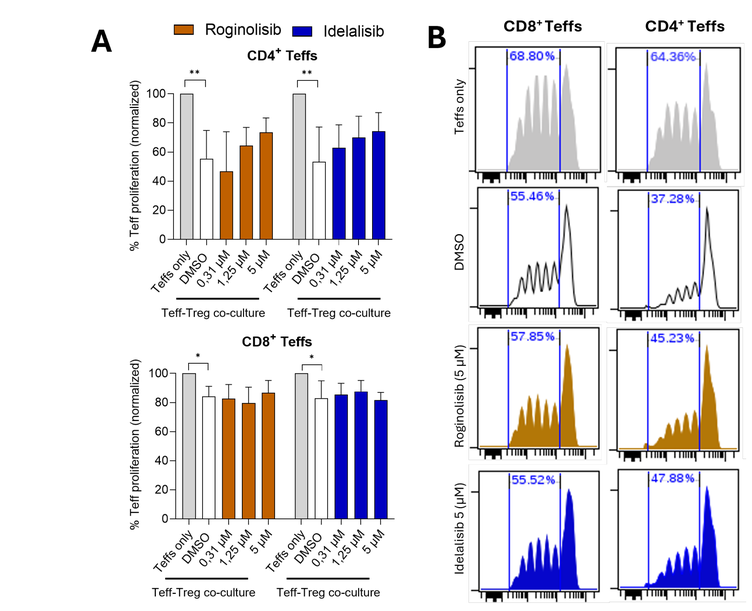


Figure 6: Isolated Tregs were stimulated with anti-CD3/2/28-coated beads and treated with DMSO or indicated concentrations of roginolisib or idelalisib for 48 hours. Next, the Tregs were washed, counted and co-cultured with isolated $CD4^+$ or $CD8^+$ T effector cells at a ratio of 1:5 for 4 days. The T effector cells were stained with CFSE CellTrace dye prior to co-culture to enable measurement of T effector cell proliferation. **A)** Mock-treated Tregs significantly suppressed T effector cell proliferation compared to T effector cells cultured in the absence of Tregs, and although both drugs had a tendency to restore $CD4^+$ T effector cell proliferation, none of the drugs restored T effector cell proliferation significantly compared to mock treated Tregs (n=3). **B)** Representative CFSE-staining.

Idelalisib, but not roginolisib, promotes conventional CD4+ T cell subsets

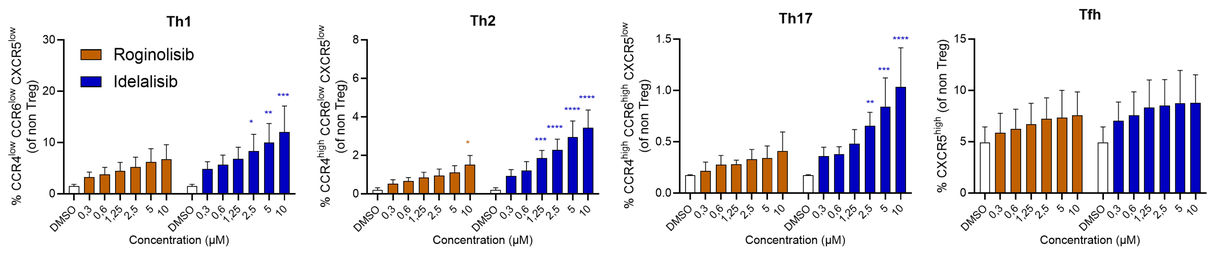


Figure 7: $CD4^+$ T cells were isolated from healthy donor blood, activated with anti-CD3/28 and treated for 48 hours with DMSO or indicated concentrations of idelalisib or roginolisib . The cells were stained with antibodies against relevant surface markers to identify the different $CD4^+$ T helper cell subsets, and analyzed by flow cytometry. Idelalisib induced a concentration-dependent increase in the percentage of $CCR4^{low}CCR6^{low}CXCR5^{low}$ Th1 cells, whereas roginolisib had no significant effect. A similar pattern was observed for $CCR4^{high}CCR6^{low}CXCR5^{low}$ Th2 cells, where idelalisib concentrations equal to or above 1,25 μM led to an increase in cell percentage, while a significant effect from roginolisib was only observed at the highest concentration of 10 μM. Furthermore, idelalisib at 2,5 μM and above promoted expansion of $CCR4^{high}CCR6^{high}CXCR5^{low}$ Th17 cells, while roginolisib again had no impact. Finally, despite a slight upward trend, neither drug significantly affected the $CXCR5^{high}$ follicular helper T cell subset (n=3).

CONCLUSIONS

- Roginolisib inhibited chronic lymphocytic leukemia (CLL) cell signaling and viability in a manner comparable to idelalisib.
- Both drugs specifically inhibited PI3K-mediated signaling in T cells, validating its on-target effects.
- Both drugs reduced T cell proliferation, but to differing extents. However, only idelalisib induced a pronounced impairment of $CD8^+$ T cell cytotoxic function at the highest concentration tested.
- Both drugs modestly reduced Treg activation and suppressive functions of $CD4^+$ T effector cells at a low Treg ratio.
- Idelalisib treatment promoted the differentiation of $CD4^+$ T cells into Th1, Th2, and Th17 subsets — a response not observed with roginolisib.

CONFLICT OF INTEREST DECLARATION

S.S.S. has received consulting fees from AstraZeneca, BeiGene and Janssen; and has received research support from BeiGene and TG Therapeutics. AB and GDC are employees at iOnctura SA. MW and DM received funding from iOnctura SA.

ACKNOWLEDGEMENTS

iONCTURA
UNIVERSITETET I OSLO
OSLO UNIVERSITETSSYKEHUS
Taskén lab
The Research Council of Norway
Cell Signaling and Immune Regulation group