# PI3K-Delta Inhibitors Induce Primary Monocyte Cytotoxicity but Do Not Alter Monocyte Differentiation

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## Background

- Chronic Lymphocytic Leukemia (CLL) is a common B-cell lympho-proliferative disorder.
- PI3K-δ inhibitors are effective therapies for CLL.
- PI3K-δ inhibitors include Idelalisib (FDA approved) and Umbralisib (TGR-1202 - in clinical studies).
- PI3K-δ inhibitors cause CLL cell apoptosis, cytotoxicity, and reduction of AKT phosphorylation *in vitro*.
- Monocyte-derived cells, also known as "nurse like cells" (NLC) are considered to be a component of the CLL lymph node microenvironment.
- Clinically, PI3K-δ inhibitors cause initial lymphocytosis thought to be due to a disrupted CLL cell – NLC interaction, with egress of CLL cells from the lymph node microenvironment.
- The direct effect of PI3K-δ inhibitors on monocytes is unknown.

## Hypothesis

PI3K-δ inhibitors induce monocyte cytotoxicity, inhibit differentiation towards M1 or M2 polarized mono-cytes, and reduce monocyte AKT phosphorylation.

## Results

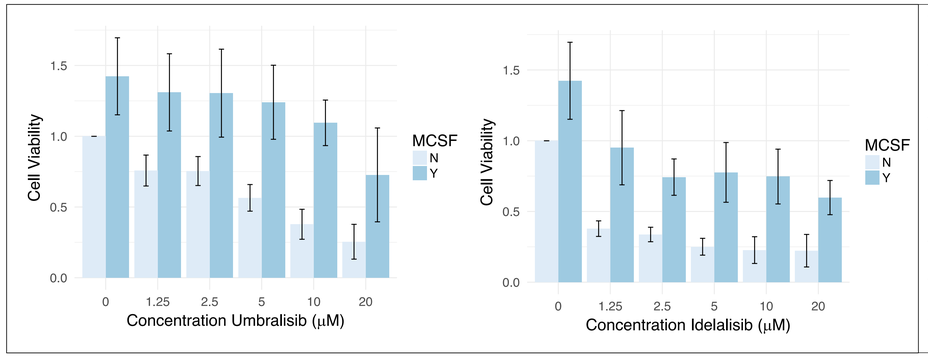
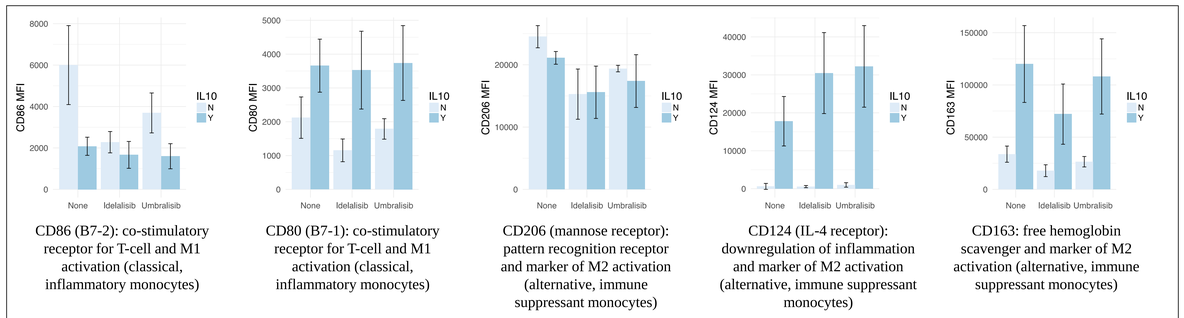



Fig 1: PI3K-δ inhibitors are cytotoxic to monocytes (Idelalisib > Umbralisib). M-CSF partially reverses cell death. Cell Viability normalized to results with no drug and no MCSF. N = 3.




CD86 (B7-2): co-stimulatory receptor for T-cell and M1 activation (classical, inflammatory monocytes)

CD80 (B7-1): co-stimulatory receptor for T-cell and M1 activation (classical, inflammatory monocytes)

CD206 (mannose receptor): pattern recognition receptor and marker of M2 activation (alternative, immune suppressant monocytes)

CD124 (IL-4 receptor): downregulation of inflammation and marker of M2 activation (alternative, immune suppressant monocytes)

CD163: free hemoglobin scavenger and marker of M2 activation (alternative, immune suppressant monocytes)

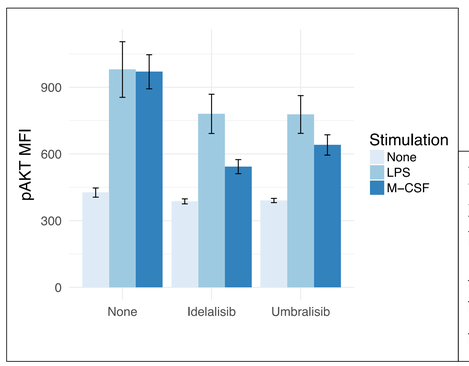



Fig 2: PI3K-δ inhibitors do not significantly or consistently affect expression of markers of monocyte differentiation. There is no significant difference between the two PI3K-δ inhibitors. N = 3.

Fig 3: AKT phosphorylation is significantly reduced by PI3K-δ inhibitors in monocytes, regardless of stimulation ($p < 0.05$). Umbralisib had significantly less inhibition of M-CSF induced AKT phosphorylation, compared to Idelalisib ($p = 0.02$), but there was no difference between PI3K-δ inhibitors with regards to inhibition of LPS induced AKT phosphorylation. N = 5.

## Methods

- Monocytes were isolated from normal donors using negative selection (RosetteSep monocyte).
- Cytotoxicity was measured using the MTS reagent. Primary purified monocytes were incubated ± M-CSF (10 ng/mL) ± PI3K-δ inhibitor (at 1.25 to 20 μM) for three days.
- Monocyte differentiation was measured using flow cytometry to measure expression of CD14, CD206, CD163, CD124, CD80, and CD86. Primary purified monocytes were incubated first with M-CSF (10 ng/mL) for three days, then washed and incubated ± IL-10 (20 ng/mL) ± PI3K-δ inhibitor (10 μM) for three days.
- AKT phosphorylation was measured using flow cytometry after whole blood incubation with LPS (50 ng/mL) or M-CSF (100 ng/mL) ± PI3K-δ inhibitor (10 μM).
- Statistical analyses were performed in the statistical environment, R.

## Conclusions

- PI3K-δ inhibitors affect signal transduction and viability, but not differentiation, of normal monocytes *in vitro*.
- There were differences noted between Idelalisib and Umbralisib with regards to the extent of cytotoxicity induced and inhibition of M-CSF induced pAKT.
- The clinical benefit and initial lymphocytosis seen with PI3K-δ inhibitors in CLL may be related in part to direct effects on monocyte-derived cells.
- Inhibition of monocyte function and/or induction of monocyte toxicity *in vivo* may suppress the innate immune system, increasing the risk of atypical infections in CLL patients taking PI3K-δ inhibitors.
- The direct effects of PI3K-δ inhibitors on monocytes suggests these drugs may have efficacy in monocytic neoplasms or in other malignancies with monocyte derived cells in the tumor microenvironment.

## References

- Burger JA et al, "Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1." Blood, 2000.
- Brown, JR et al, "Idelalisib, an inhibitor of phosphatidylinositol 3-kinase p110δ, for relapsed/refractory chronic lymphocytic leukemia." Blood. 2014.