**Background**

- Chronic Lymphocytic Leukemia (CLL) is a common B-cell lymphoproliferative disorder.
- PI3Kδ inhibitors are effective therapies for CLL.
- PI3Kδ inhibitors include Idelalisib (FDA approved) and Umbralisib (TGR-1202 - in clinical studies).
- PI3Kδ 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, PI3Kδ 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 PI3Kδ inhibitors on monocytes is unknown.

**Hypothesis**

PI3Kδ inhibitors induce monocyte cytotoxicity, inhibit differentiation towards M1 or M2 polarized monocytes, and reduce monocyte AKT phosphorylation.

**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) ± PI3Kδ 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) ± PI3Kδ 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) ± PI3Kδ inhibitor (10 μM).
- Statistical analyses were performed in the statistical environment, R.

**Results**

- PI3Kδ inhibitors are cytotoxic to monocytes (Idelalisib = Umbralisib). M-CSF partially reverses cell death. Cell Viability normalized to results with no drug and no M-CSF. N = 3.
- PI3Kδ inhibitors do not significantly or consistently affect expression of markers of monocyte differentiation. There is no significant difference between the two PI3Kδ inhibitors. N = 3.
- AKT phosphorylation is significantly reduced by PI3Kδ 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 PI3Kδ inhibitors with regards to inhibition of LPS induced AKT phosphorylation. N = 5.

**Conclusions**

- PI3Kδ 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 PI3Kδ 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 PI3Kδ inhibitors.
- The direct effects of PI3Kδ 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**