Survival and proliferation of chronic lymphocytic leukemia (CLL) cells is favored by the essential role of the microenvironment (ME) that is responsible, at least in part, for disease progression and drug resistance. Hence, we planned to evaluate and predict the efficacy of therapeutic compounds in vivo, by reproducing in a co-culture system the different microenvironmental components that enable B cells to survive and proliferate, mimicking in particular the lymph node microenvironment where most of the crucial events of the pathogenesis of CLL occur.

**RESULTS**

Cryopreserved peripheral blood (PB) mononuclear cells from CLL patients were tested with the EnvTech® proprietary automated flow cytometry-based platform. Different components/conditions have been evaluated to reproduce the ME and induce proliferation and survival of CLL cells: (i) 3 backbone stimulations: CD40L+CpG, CD40L+IL2, CpG+IL2; (ii) “Native Environment”, defined as the plasma & erythrocytes/glycocalyx fraction of a Ficoll gradient; (iii) the stroma cell line H5S, added at different ratios (1:10 or 1:100); (iv) either human or bovine fetal serum (at 10 or 20% total volume); (v) stimulatory B cell factors, including IL-21, soluble CD40L, BAFF, and B cell receptor stimulation (anti-IG).

**METHODS**

![Native Environment from Fresh Sample](image)

**Figure 1.** Addition of Native Environment (right, blue) on Cryopreserved CLL samples (left, red). After Ficoll, plasma layer is stored at -80°C and RBC at 4°C with CPDA solution. Both fractions are mixed in a 1:1 proportion and added to the thawed CLL samples.

**BACKGROUND AND AIM**

**Figure 2.** Each blue circle represents the median value for proliferation and apoptosis of 5 CLL samples for all the 36 conditions tested. Best conditions are indicated in the panel at 96 h. (NPB: NE from normal peripheral blood; CLL: NE from CLL patients)

**Figure 3.** 4 progressive CLL samples were seeded with and without H5S stromal cells and CpG+IL2. NE was derived from a mixing of the Ficoll fractions of 6 NPB or 6 CLL PB samples (5 stable and 1 progressive).

**Figure 4.** 26 different cytokine conditions were tested on 20 progressive CLL frozen samples

**Figure 5.** Representative case of a proliferation assay using CpG+IL2 + H5S (1:100) + HS10% + CLL NE (pooled samples). Red color: CLL cells in the absence of any drug at 96 h from incubation; Grey color: CLL cells in the presence of Idelalisib show blocked proliferation.

**Idelalisib and Ibrutinib show potent activity in proliferating CLL cells**

**Ibrutinib and Idelalisib: novel antiproliferative mechanism of action**

**CONCLUSION**

We here report a novel ex vivo assay that incorporates TME stimuli, thus more accurately simulating in vivo interactions and enabling high-throughput pharmacological characterization under physiological conditions. Proliferating cells are more sensitive to Idelalisib. This assay demonstrated an anti-proliferative activity for Idelalisib and Ibrutinib that may explain the efficacy of both drugs in patients, identifying those more likely to respond to either Idelalisib or Ibrutinib treatment.

**RESULTS**

| Cpd     | NE CLL | NE NPB | Control Cpd (2) |
|---------|--------|--------|----------------|--------|--------|---------------|
| viability |        |        |                |        |        |               |
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