EX VIVO LYMPH NODE NATIVE MICROENVIRONMENT ASSAY SHOWS NOVEL ANTIPROLIFERATIVE ACTIVITY FOR IDELALISIB AND IBRUTINIB ON CLL CELLS

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INTRODUCTION AND AIM

Survival and proliferation of chronic lymphocytic leukemia (CLL) cells is favoured by the essential role of the microenvironment (ME) that is similarly responsible at least in part for drug resistance and progression of the disease. For these reasons, in order to evaluate and predict the efficacy of therapeutic compounds *in vivo,* it is crucial to reproduce in a co-culture system all the different microenvironmental components that enables B cells to survive and proliferate mimicking in particular the lymph node microenvironment where most of the crucial events of the pathogenesis of CLL are thought to occur.

METHODS

To this purpose, cryopreserved peripheral blood (PB) mononuclear cells from CLL patients in need of treatment were utilized and tested with the Exvitech® proprietary automated flow cytometry-based platform. Different components have been added and compared to reproduce the ME and induce proliferation and survival of CLL cells: (i) 3 backbone stimulations: CD40L+CpG, CD40L+IL21, CpG+IL2; (ii) "Native Environment", defined as the plasma & erythrocyte/granulocyte fraction of a Ficoll gradient: (iii) the stroma cell line H5S, added at different ratios (1:10 or 1:100); (iv) both human and 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).

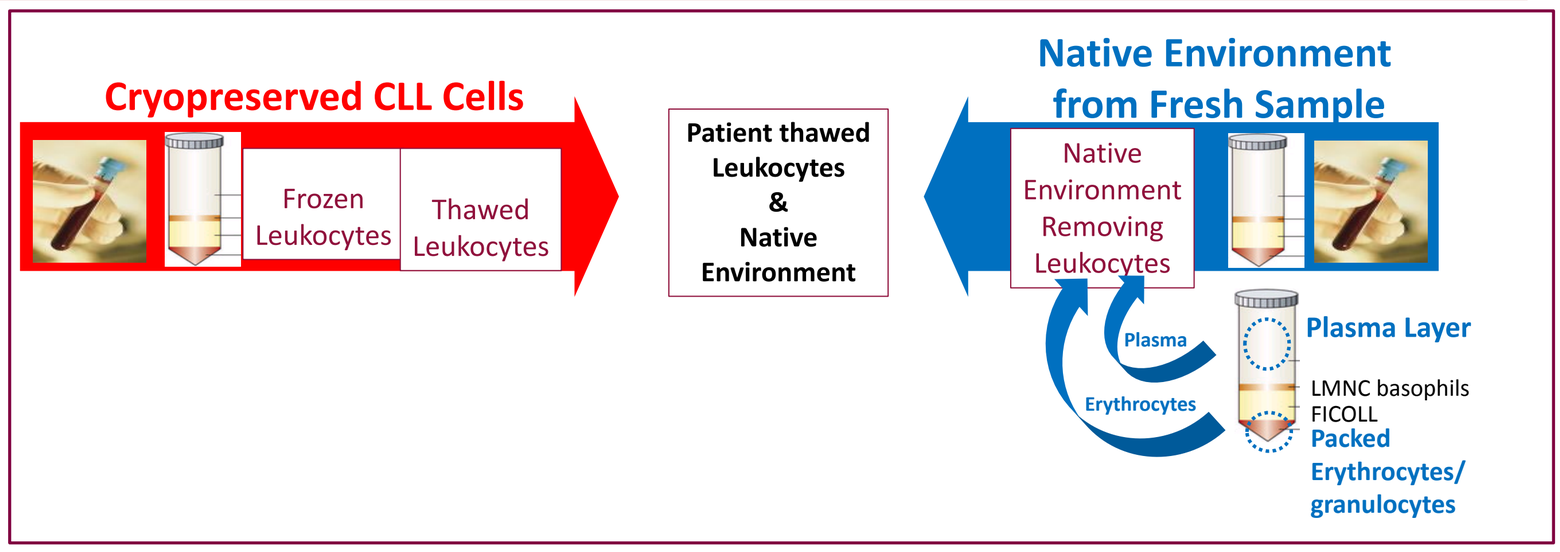


Figure 1: Addition of Native Environment (right, green) on Cryopreserved CLL samples (left, red). After Ficoll, plasma layer is stored at -80ºC and RBC at 4ºC with CPDA solution. Both fractions are mixed in a 1:1 proportion and added to the thawed CLL samples.

RESULTS

Assay optimization reveals HS5 cells and CpG+IL2 as the best condition

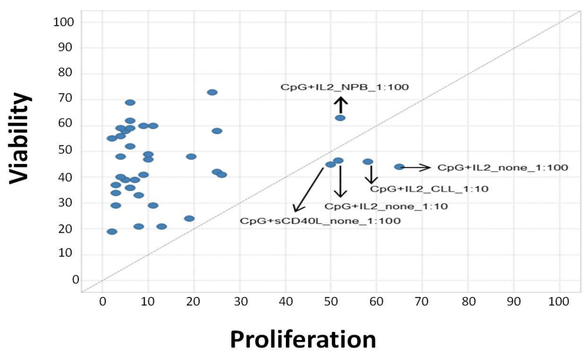


Figure 2: Each blue circle represents the median value for proliferation and apoptosis of 5 CLL samples for all the 36 conditions tested. Best conditions are indicated in the panel at 96 h. (NPB: NE from normal peripheral blood; CLL: NE from CLL patients)

Native Environment from CLL samples improves proliferation

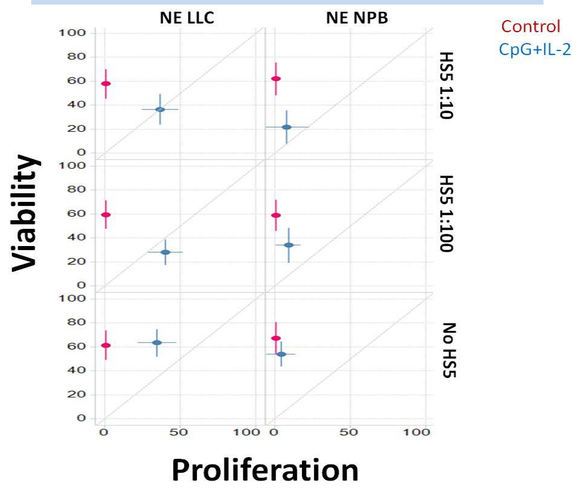


Figure 3: 4 progressive CLL samples were seeded with and without HS5 stromal cells and CpG+IL2. NE was derived from a mixing of the Ficoll fractions of 6 NPB or 6 CLL PB samples (5 stable and 1 progressive).

CpG+IL-2 + HS5 (1:100) + HS10% + CLL NE (pooled samples) is the best combination

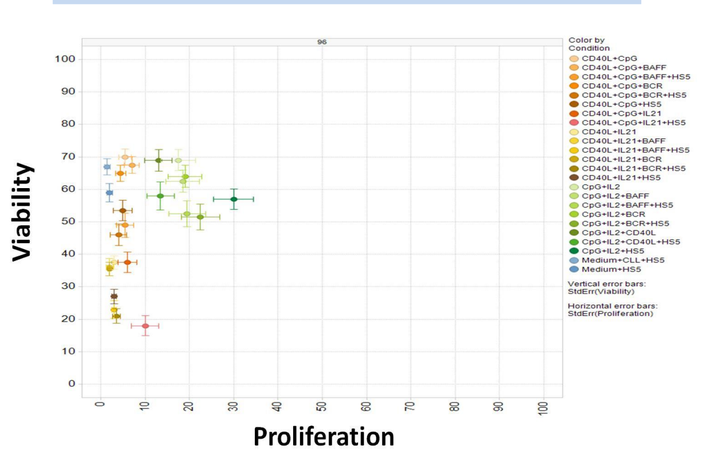


Figure 4: 26 different cytokine conditions were tested on 20 progressive CLL frozen samples.

Idelalisib is a potent antiproliferative compound

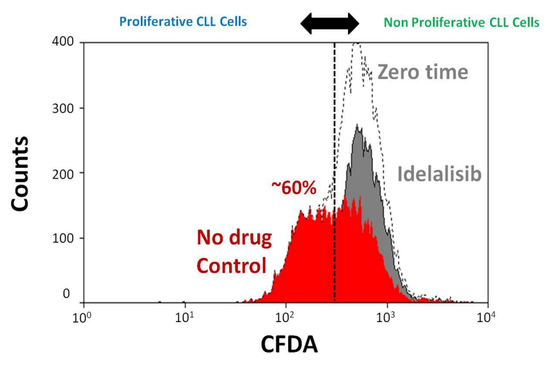
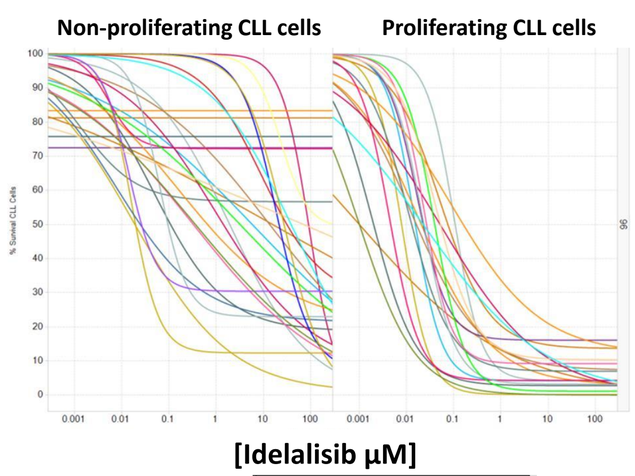
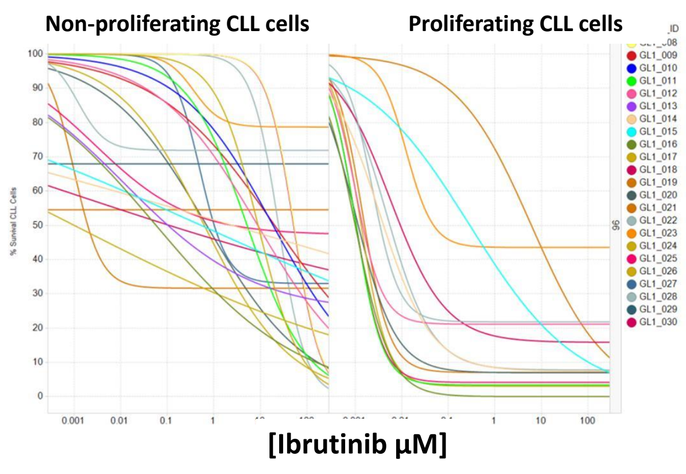


Figure 5: Red color: CLL cells in the control well; Grey color: CLL cells under a high dose of Idelalisib show blocked proliferation (they are alive, not apoptotic).



	Live Tumor Cells	
	Proliferative	Non-Proliferative
N	19	29
Avg(EC50) µM	0,03	12,49
StdDev(EC50) µM	0,03	22,48
Avg(Emax) %	5,19	20,52
StdDev(Emax) %	5,02	23,82



	Live Tumor Cells	
	Proliferative	Non-Proliferative
N	16	25
Avg(EC50) µM	0,55	28,28
StdDev(EC50) µM	1,6	99,41
Avg(Emax) %	8,87	17,38
StdDev(Emax) %	11,71	24,15

Figure 6: 29 CLL frozen samples (color coded) were tested at 96h with the (CpG+IL2 + H5S(1:100) + CLL-NE) assay condition, measuring dose response curves for Idelalisib and Ibrutinib in both the Non-Proliferating (left panel) and Proliferating (middle panel) fractions. Both compounds show potent nM activity in proliferating cells vs poor µM activity in non-proliferating cells

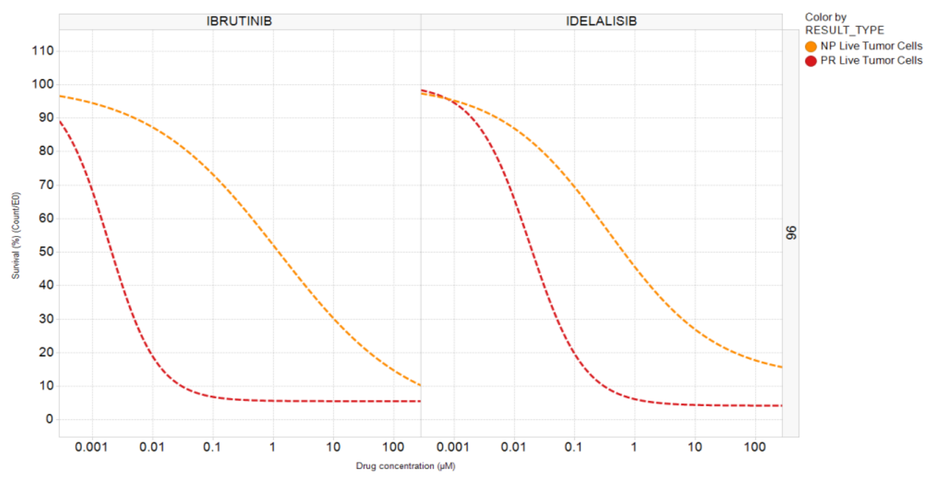


Figure 7: Median dose response curves from Figure 6 for Idelalisib and Ibrutinib in both the Non-Proliferating (orange) and Proliferating (red) fractions. Both compounds show a previously unknown antiproliferative mechanism of action.

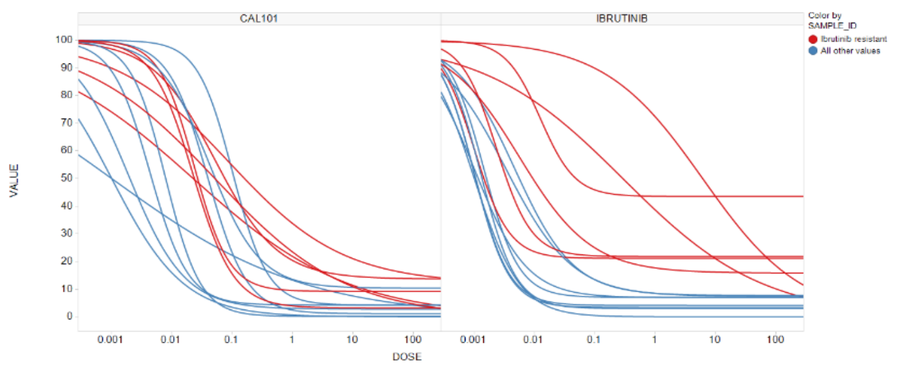


Figure 8: Potentially resistant (or less sensitive) Ibrutinib patient samples (red) behave more normal for Idelalisib. Assay could help identify patients more suited for Idelalisib vs Ibrutinib treatment

CONCLUSION

We report a novel *ex vivo* assay that enables high-throughput pharmacological characterization incorporating Lymph Node Microenvironment stimuli and thereby more accurately simulating *in vivo* interactions. This assay has revealed an unknown anti-proliferative mode of action for Idelalisib and Ibrutinib, drugs that interfere with the CLL Microenvironment.