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 Exvitex® 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+Cpg6, CD40L+H2L2, Cpg6+H2L2; (ii) “Native Environment”, defined as the plasma & erythrocyte/granulocyte fraction of a Ficoll gradient; (iii) the stroma cell line H55, 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).

RESULTS

Figure 1: Addition of Native Environment (right, green) 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.

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 H55 stromal cells and Cpg6+H2L2. 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: Red color: CLL cells in the control well. Grey color: CLL cells under a high dose of Idealalisib show blocked proliferation (they are alive, not apoptotic).

Figure 6: 29 CLL frozen samples (color coded) were tested at 96h with the (Cpg6+H2L2 + HSS:1:100) + CLL-NF assay condition, measuring dose response curves for Idealalisib and Ibrutinib in both the Non-Proliferating (left panel) and Proliferating (middle panel) fractions. Both compounds show potent mpa activity in proliferating cells vs poor mpa activity in non-proliferating cells.

Figure 7: Median dose response curves from Figure 6 for Idealalisib 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 Idealalisib. Assay could help identify patients more suited for Idealalisib 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 Idealalisib and Ibrutinib, drugs that interfere with the CLL Microenvironment.

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