

Characterization and integration of mantle cell lymphoma microenvironments are determinant for the development of rational targeted therapies

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AIMS

The aggressive biological behavior of mantle cell lymphoma (MCL) and its short response to current treatment highlight a great need for better rational therapy. Recently it has been demonstrated that tumor microenvironment strongly influences drug resistance in MCL cells. In the present study, we developed an *ex vivo* model that mimics *in situ* molecular profiles and allows the development of new approaches by integrating the key role of the microenvironment.

METHODS

Primary cells were obtained after informed consent from MCL patients treated at the Department of Clinical Hematology from the University Hospital of Nantes and were coculture *ex vivo* with MSC, CD40L+ cells and MCL specific cytokine cocktail (IL6, IL10, BAFF, IGF1). Transcriptomes of primary MCL cells were determined using RNA-seq (Illumina) technology. BH3 profiling was performed using the BH3-mimetic Venetoclax (VNT, 2-20 mM), the BIM BH3 peptide (0.05 mM), and engineered HRK*BH3 (5mM) and Noxa*BH3 (5mM) peptides.

RESULTS

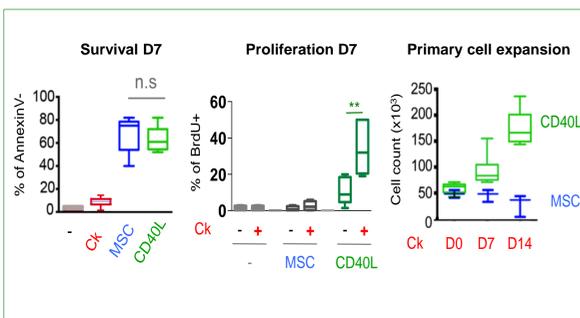


Fig 1. Despite a significant level of the proliferation index Ki67 in lymph nodes, we did not detect any proliferating peripheral blood MCL cells, suggesting a major role of the tumor ecosystem. To determine interactions that could support survival and proliferation, primary MCL cells were cocultured in several conditions *ex vivo*. Primary MCL cells alone or with cytokines (Ck) do not survive on long term *ex vivo* culture. In contrast, both MSC or CD40L+ cells support their survival, but only CD40L+ cells are able to reactivate the cell cycle, the proliferation being greatly enhanced in the presence of cytokines. Our «CD40L+Ck» model allowed survival, proliferation and functional studies in all primary samples tested (n>30) and even expansion for the most proliferating ones.

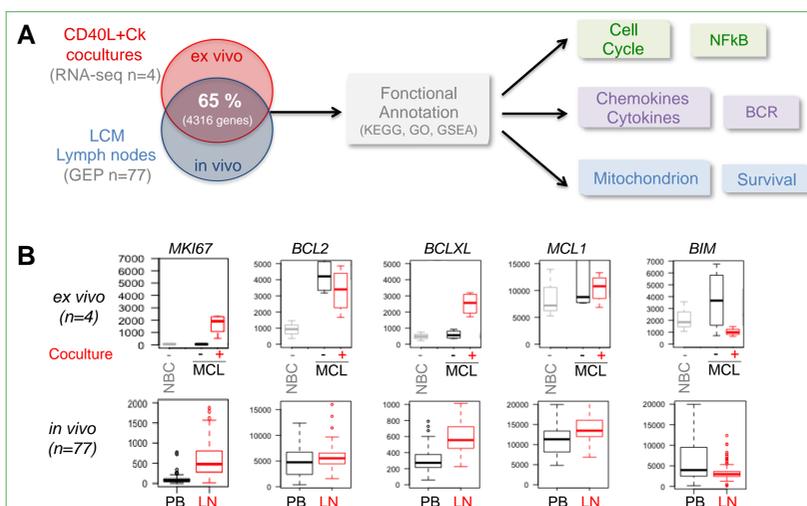


Fig 2. (A) To confirm the relevance of the «CD40L+Ck» model, we compared the transcriptome of primary MCL cells in coculture *ex vivo* to MCL cell *in situ* in the lymph nodes (LN). We observed that more than 65% of the genes induced in coculture were also induced in LN compared to peripheral blood (PB). Our model recapitulates molecular signatures that are characteristic of MCL such as cell cycle, BCR, NFkB/NIK and survival, confirming the relevance of the coculture. (B) We further studied the coculture-induced regulation of genes belonging to the survival signature and especially the druggable Bcl2 family. The major regulation was an increase in expression of BCLXL associated with a downregulation of BIM.

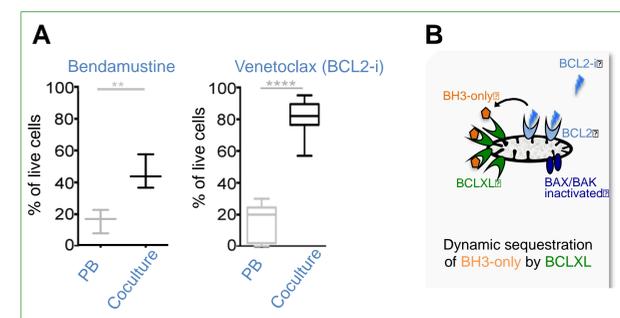


Fig 3. (A) Unbalance in Bcl2 family have consequences, especially in drug resistance and eventually relapses. Indeed, whereas peripheral blood (PB) MCL cells are highly sensitive to alkylating agents (Bendamustine) or BCL2 inhibitor (Venetoclax), cells in coculture are more resistant. (B) To understand the molecular mechanisms involved, we used the functional BH3-profiling assay. We then observed that, whereas PB MCL cells are dependent on BCL2 for survival, the coculture was responsible for loss of mitochondrial priming. This loss of priming was the consequence of a dynamic sequestration of the pro-apoptotic BH3-only Bim by BCLXL after its release from BCL2 upon drug pressure. We then concluded that targeting BCLXL could be an efficient strategy to sensitize MCL cells in their protective niches.

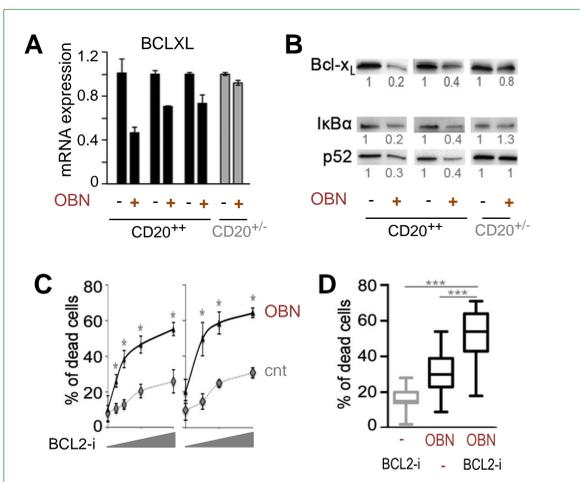


Fig 4. (A) We observed that the type II anti-CD20 Obinutuzumab (OBN) was able to specifically downregulate BCLXL in CD20⁺ primary MCL cells. (B) BCLXL downregulation was associated to an inhibition of both classical and alternative NFkB pathways. (C-D) As expected, OBN-dependent BCLXL down-regulation sensitizes primary MCL cells to the BCL2-i Venetoclax.

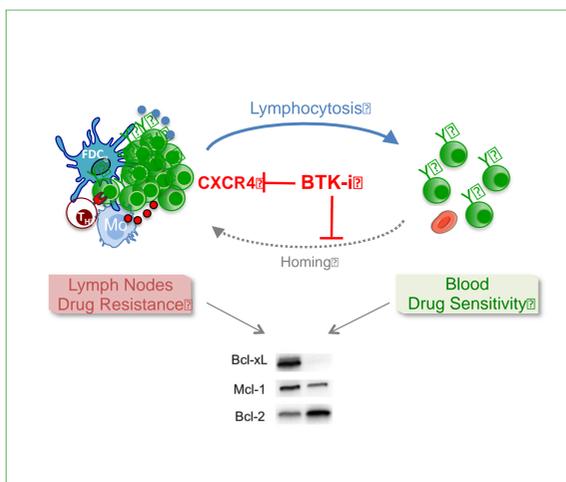


Fig 5. An alternative strategy could come from the BTK-i Ibrutinib, which interferes with chemokine signalings, mainly CXCR4 in MCL. Ibrutinib inhibits the homing of MCL cells in the bone marrow or lymph nodes and a consequent lymphocytosis can be observed *in vivo* in the first weeks of treatment. Cells that egress in the PB have a molecular profile BCLXL^{LOW} BCL2^{HIGH}.

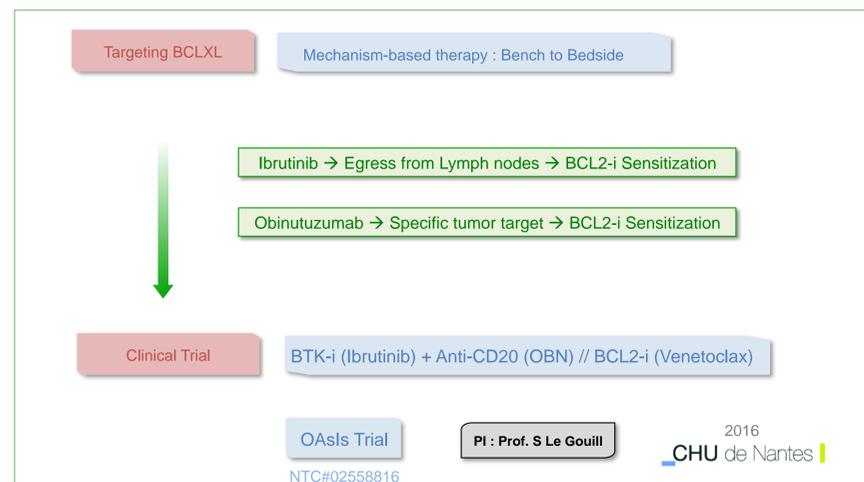


Fig 6. We hypothesized that targeting BCLXL could increase treatment efficacy. Using our coculture model, we developed efficient targeted strategies (i.e: BTK inhibitor, Type II anti-CD20), which counteract BCLXL overexpression and overcome drug resistance in primary cells *ex vivo*. This strategy should target cells protected into their protective niches and our ongoing OASIs Trial (NTC02558816) will rapidly determine *in vivo* efficacy in MCL.

CONCLUSIONS

- Aggressive proliferation of MCL is associated to lymphoid signals (CD40L+Ck)
- Microenvironment-dependent modulation of the Bcl-2 family lead to survival
- Drug resistance is the consequence of a loss of mitochondrial priming

The lymphoma ecosystem must be integrated into future mechanism-based therapeutic strategies

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