Poster #: 1302

XXI IWCLL International Workshop on CLL

Pevonedistat Enhances the Cytotoxic Effect of Rohinitib on B Cells of Chronic Lymphocytic Leukemia

Jose Luis Castaño[1], Victor Arenas[1], Daniela Vallejo[1], Juan José Domínguez[2], Lucrecia Yáñez[2] and Carlos Pipaón[1]

1. Instituto de investigación Marques de Valdecilla (IDIVAL)

2. Servicio de Hematología, Hospital Universitario Marques de Valdecilla

OBJECTIVES

- Study the effect of the combined inhibition of RNA translation and Neddylation on CLL.
- Analyze its mechanism to provide information on the molecular causes of this pathology.

CONCLUSIONS

- The combination of Pevonedistat and Rohinitib shows promising synergistic effects inducing cell death in B lymphocytes from CLL patients.
- The cytotoxic effect of the combination of Pevonedistat and Rohinitib seems to be specific of CLL, and it is not mediated by Ubiquitination.
- The process of translation is not altered when inhibiting the Neddylation in CLL.
- The Neddylation levels can be reduced in CLL by the inhibition of EIF4A1.
- The combination of Pevonedistat and Rohinitib in CLL produce alterations in the expression level of apoptosis related genes as BCL2 and TP53.

Hematología Valdecilla Servicio de Hematología Hospital Universitario Marqués de Valdecilla

Valdecilla Instituto de Investigación Sanitaria IDIVAL

HEMMOL Hematología Molecular

INTRODUCTION

CLL is the most prevalent leukemia in adults. There is not a cure for the CLL, and some patients may develop a resistant effect to the current treatments that improve their condition[1].

Previous studies tried to find the cause of CLL, but it is still not clear. There are some altered transversal processes that may play an important role in the development of CLL[2]:

1- There are discrepancies between the mRNA and protein levels of certain genes in CLL cells. Recently, overexpression of the translation initiation factor eIF4A1 has been described in CLL and other tumors, and how its inhibition blocks the dependence of these tumors on the translation of oncogenes[3].

2- CLL cells show an elevation of ubiquitin-like post-translational modifications (UBL-PTM), among which the role of Neddylation in its pathogenesis has been highlighted. Neddylation modulates the homeostasis of a wide range of proteins, as it is necessary for their degradation by the ubiquitin-proteasome system[4].

METHODS

We analyzed the cytotoxicity of a translation inhibitor (Rohinitib) and a Neddylation inhibitor (Pevonedistat) on B cells from CLL patients treated ex vivo, using analysis of 7-AAD staining by flow cytometry, as well as by oxidation of tetrazolium salts (XTT). We studied the translation process analyzing an OPP staining by flow cytometry. We conducted an analysis of the changes in gene expression produced by RNA seq and the accumulation of key proteins by western blot.

RESULTS

Both Pevonedistat and Rohinitib separately induced a significant increase in the death of B cells from a cohort of CLL patients treated ex vivo (Figure 1A). However, the combined treatment with both drugs enhanced their effect synergistically (Figure 1B) and dose response (Figure 1C), peaking 80% after 72 hours (Figure 1D). The induction of cell death by this combined treatment was much lower in B lymphocytes obtained from healthy donors (Figure 1A). Moreover, this effect appears to be specific to direct NEDDylation, as the same synergy is not observed with the ubiquitination inhibitor, TAK-243 (Figure 2A). This cell death sems to be cause by the apoptosis machinery as we can observe an increase in the degestion of PARP (Figure 2B) and an increase in the number of events in the sub-G0 peak, (Figure 2C). Furthermore, this synergy does not occur in multiple myeloma cell lines (Figure 2D).

To decipher the mechanism of this synergistic effect, we carried out a series of molecular determinations. First, we studied the possible interrelation between the elevated level of UBL-PTM and the increase in translation in B-CLL cells. A massive screening in patient samples showed that multiple translation factors exhibit an altered UBL-PTM modification pattern compared to that of circulating B lymphocytes from healthy donors (Figure 3A). Treatment with Pevonedistat did not produce changes in the accumulation of eIF4A1 or its natural inhibitor, PDCD4 (Figure 3B) neither changes in the global translation (Figure 3C). On the contrary, treatment with Rohinitib reduced the Neddylation of proteins (Figure 4A) and the accumulation of two members of the Neddylation pathway (Figure 4B). Finally, we conducted a study of the variations in the transcriptome of 5 CLL patients in response to treatment with Rohinitib and Pevonedistat (Figure 5A). Our data showed a cooperative effect of these drugs in the inhibition of BCL2 and the activation of TP53, suggesting their mediation in the synergistic induction of apoptosis (Figure 5B).

Figure 1. Synergic effect of Pevonedistat and Rohinitib

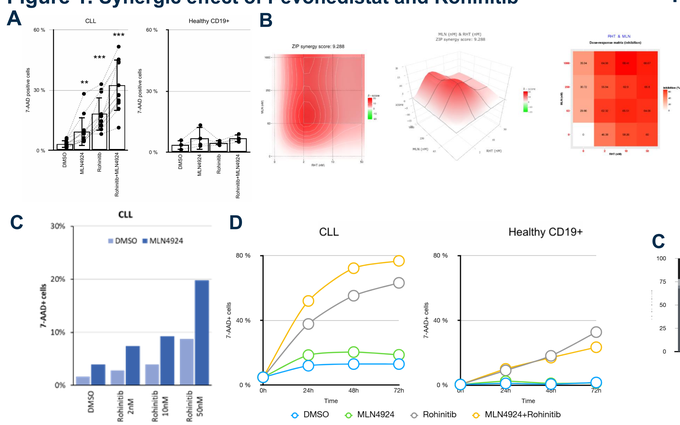


Figure 2. Specificity of the cytotoxic effect

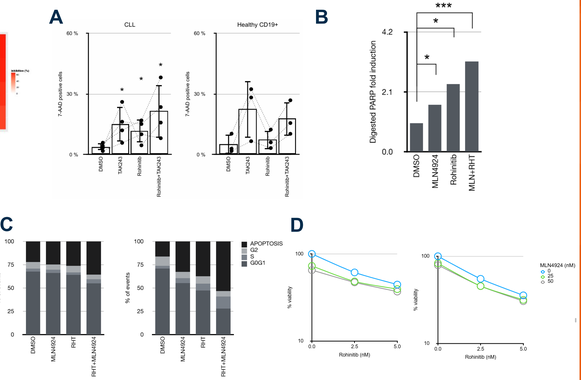


Figure 3. Effect of Neddylation over translation

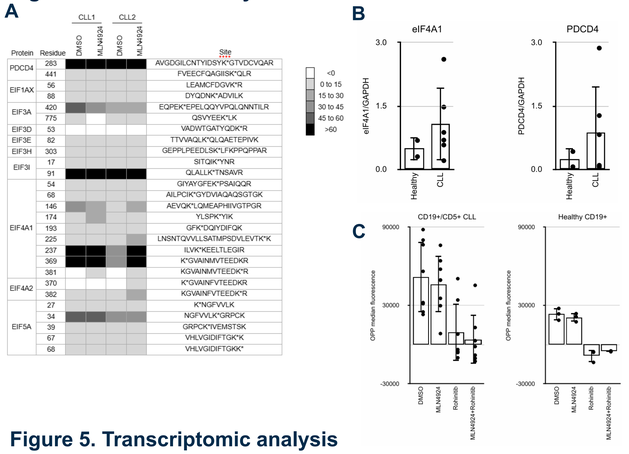


Figure 4. Effect of translation over Neddylation

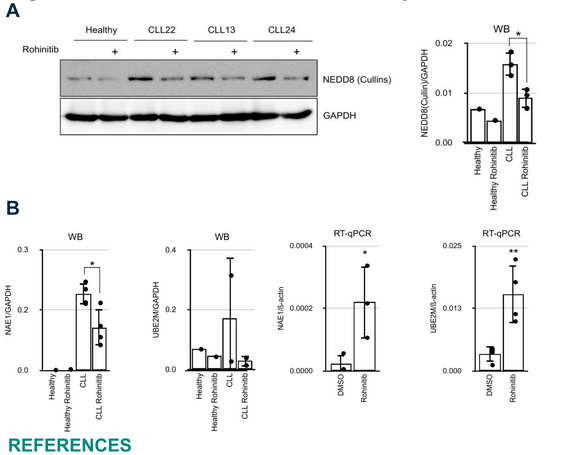
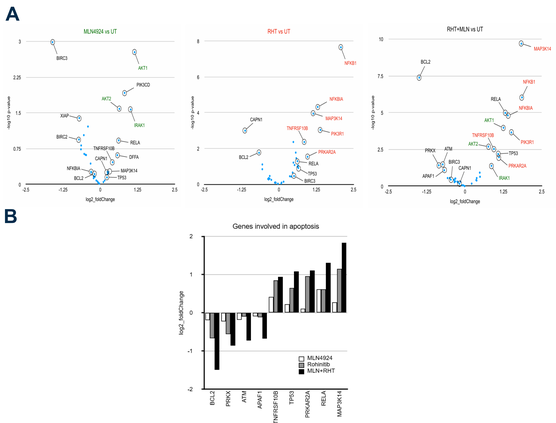


Figure 5. Transcriptomic analysis



REFERENCES

1. Odetola O, Ma S. Relapsed/Refractory Chronic Lymphocytic Leukemia (CLL). Curr Hematol Malig Rep. 2023 Oct;18(5):130-143. doi: 10.1007/s11899-023-00700-z. Epub 2023 Jun 6. PMID: 37278884; PMCID: PMC10242240.

2. Paul, P., Stüssi, G., Bruscaggin, A., & Rossi, D. (2022). Genetics and epigenetics of CLL. *Leukemia & Lymphoma*, *64*(3), 551–563. https://doi.org/10.1080/10428194.2022.2153359

3. Largeot A, Klapp V, Viry E, Gonder S, Fernandez Botana I, Blomme A, Benzarti M, Pierson S, Duculty C, Marttila P, Wierz M, Gargiulo E, Pagano G, An N, El Hachem N, Perez Hernandez D, Chakraborty S, Ysebaert L, François JH, Cortez Clemente S, Berchem G, Efremov DG, Dittmar G, Szpakowska M, Chevigné A, Nazarov PV, Helleday T, Close P, Meiser J, Stamatopoulos B, Désaubry L, Paggetti J, Moussay E. Inhibition of MYC translation through targeting of the newly identified PHB-eIF4F complex as a therapeutic strategy in CLL. Blood. 2023 Jun 29;141(26):3166-3183. doi: 10.1182/blood.2022017839. PMID: 37084385; PMCID: PMC10646824.

4. Paiva C, Godbersen JC, Rowland T, Danilova OV, Danes C, Berger A, Danilov AV. Pevonedistat, a Nedd8-activating enzyme inhibitor, sensitizes neoplastic B-cells to death receptor-mediated apoptosis. Oncotarget. 2017 Mar 28;8(13):21128-21139. doi: 10.18632/oncotarget.15050. PMID: 28177892; PMCID: PMC5400571.

ACKNOWLEDGMENTS

Thanks to Patricia Maiso´s laboratory for testing the combination of inhibitors in their Multiple Myeloma models

DISCLOSURES

The authors declare no conflicts of interest

CONTACT

Jose Luis Castaño: jose.castano@idival.org

Carlos Pipaón: carlos.pipaon@scsalud.es