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Therapeutical potential of Brusatol in CLL patients in monotherapy and in combination with ibrutinib and venetoclax

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OBJECTIVES

- This study evaluates the impact of NRF2 pathway inhibition in CLL patient cells using brusatol in monotherapy and in combination with conventional therapy, namely ibrutinib and/or venetoclax.

CONCLUSIONS

- Our findings highlight the BRU potential as a novel therapeutic strategy in CLL, particularly for patients with intolerance, as well as patients with poor prognosis molecular features who often experience inferior responses to current therapies.
- Further studies, including in vivo models and clinical trials, are needed to further investigate BRU's safety, toxicity and efficacy in CLL.

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INTRODUCTION

- Oxidative stress (OS) plays a crucial role in CLL development, progression and resistance to therapy. Nuclear factor erythroid 2-related factor 2 (NRF2), a major oxidative stress regulator, is deregulated in CLL cells.
- Current treatment strategies targeting BCR and BCL-2 are associated with prolonged progression free survival. However, resistance mechanisms, particularly mutations in Bruton tyrosine kinase (BTK) and in BCL-2, pose significant therapeutic challenges.
- Novel strategies, such as NRF2 modulation with brusatol (BRU), an enhancer of ubiquitination and degradation of NRF2, may offer alternative therapeutic approaches.

METHODS

- For this proposal, peripheral blood mononuclear cells from 27 CLL patients (pts) were isolated by ficol gradient and cultured in the absence (controls, CTL) as well as with different concentrations of BRU (5, 10, 25, 50, 100 nM), venetoclax, a BCL-2 inhibitor (VEN 2.5μM) and ibrutinib, a BTK inhibitor (iBTK) (IBR 10μM). The cells were also exposed to BRU in association with VEN or IBR or both.
- Cell viability was assessed using the Fluorometric Microculture Cytotoxicity Assay (FMCA) at 24h and 48h.
- Cell death was assessed by flow cytometry at 24h the using annexin V assay.
- Statistical analysis was performed using GraphPad Prism software, version 9.5 Windows (GraphPad Software, USA).

RESULTS

- We included 27 pts, 63% (n=17) were Binet A, 3,7% (n=1) Binet B and 33,3% (n=9) Binet C. Eight pts had immunoglobulin heavy chain variable region (IGHV) unmutated (in 10 pts analysed), 3 pts harboured del(17p) (in 12) and 2 pts were TP53 mutated (in 14).
- We observed that, in monotherapy, BRU induced a reduction in cell survival in a dose, time and Binet stage dependent manner.
  - IC50 for Binet A+B+C at 48h was 115nM.
  - IC50 for Binet B+C pts at 48h was 62nM, compared to IC50 for Binet A pts, which was 173nM
- We observed that CLL cells with adverse prognosis molecular markers (TP53 mutation, del(17p) or unmutated IGHV) were 4,32 times more sensitive to BRU at 48h when compared to the favourable prognosis pts.
  - IC50 at 48h of 83nM and 359nM, respectively.
- We observed that BRU alone induced CLL cell death mainly by apoptosis (p<0.001). BRU was less toxic to normal B and T cells, suggesting a selective cytotoxicity profile
- Moreover, when BRU was used in therapeutic combination, dual and triple therapies were not statistically superior to BRU in monotherapy (p≥0.05), at least in the FMCA analysis, whereas in the cell death analysis, these combinations significantly increased apoptosis (p<0.001). However, they also increased apoptosis in normal cells, raising concerns about toxicity.

Figure 1. BRU's dose-response curves, according to Binet staging, combination therapy and molecular markers

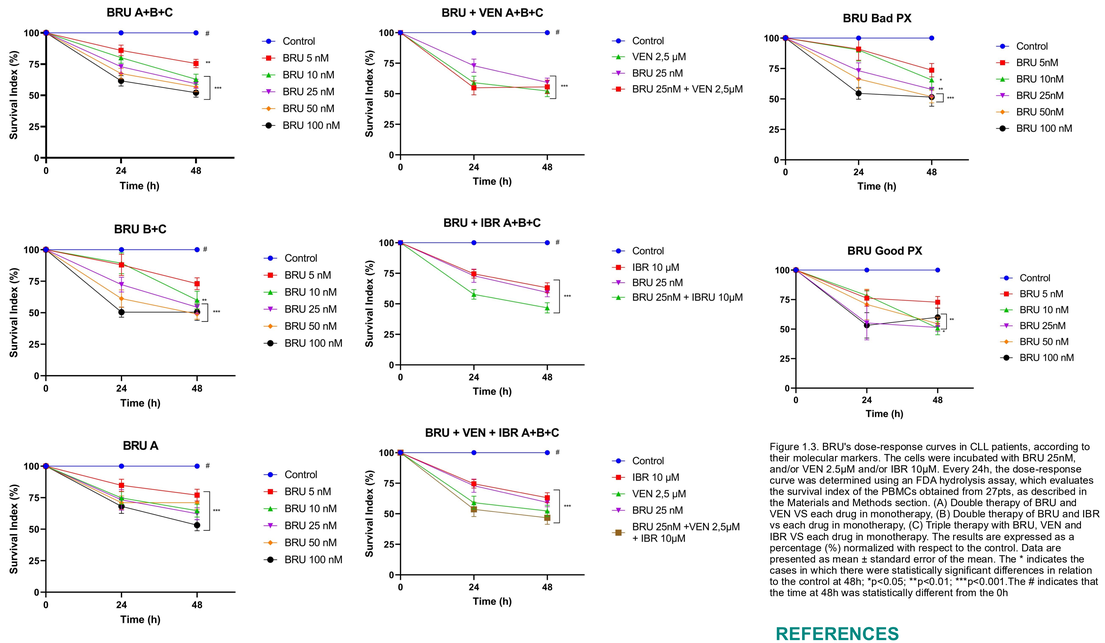
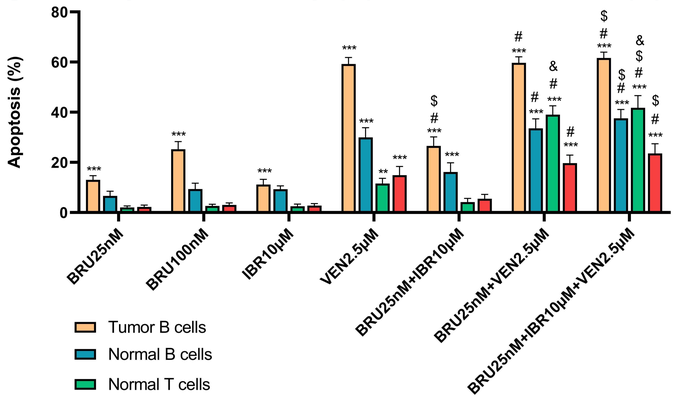


Figure 1.3. BRU's dose-response curves in CLL patients, according to their molecular markers. The cells were incubated with BRU 25nM, and/or VEN 2.5μM and/or IBR 10μM. Every 24h, the dose-response curve was determined using an FDA hydrolysis assay, which evaluates the survival index of the PBMCs obtained from 27pts, as described in the Materials and Methods section. (A) Double therapy of BRU and VEN VS each drug in monotherapy, (B) Double therapy of BRU and IBR vs each drug in monotherapy, (C) Triple therapy with BRU, VEN and IBR VS each drug in monotherapy. The results are expressed as a percentage (%) normalized with respect to the control. Data are presented as mean ± standard error of the mean. The * indicates the cases in which there were statistically significant differences in relation to the control at 48h; *p<0.05; **p<0.01; ***p<0.001.The # indicates that the time at 48h was statistically different from the 0h

Figure 1.1. BRU's dose-response curves in CLL patients, according to their Binet staging (A, B, C).The cells were incubated in the absence and presence of increasing concentrations of BRU (5nM, 10nM, 25nM, 50nM and 100nM) for 48h. At 24- and 48-hour timepoints, survival index (SI) of the PBMCs obtained from 27 patients was determined, as described in the Materials and Methods section. (A) Total-CLL population, (B) Patients in Binet B and C stage, (C) Patients in Binet A stage. The results are expressed as a percentage (%) normalized with respect to the control. Data are presented as mean ± standard error of the mean. The * indicates the cases in which there were statistically significant differences in relation to the control at 48h; *p<0.05; **p<0.01; ***p<0.001.The # indicates that the time at 48h was statistically different from the 0h.

Figure 1.2. Dose-response curves of BRU, VEN and/or IBR. The cells were incubated in the absence and presence of increasing concentrations of BRU (5nM, 10nM, 25nM, 50nM and 100nM) for 48h. The dose-response curve was determined using an FDA hydrolysis assay, which evaluates the survival index of the PBMCs obtained from 27pts at 24h and 48h, as described in the Materials and Methods section. (A) Adverse prognosis samples (BRU Bad PX - who harboured either a TP53 mutation, del(17p) or IGHV non-mutated status) (B) Favourable prognosis samples (Good PX - without these adverse markers). The results are expressed as a percentage (%) normalized with respect to the control. Data are presented as mean ± standard error of the mean. The * indicates the cases in which there were statistically significant differences in relation to the control at 48h; *p<0.05; **p<0.01; ***p<0.001.The # indicates that the time at 48h was statistically different from the 0h.

Figure 2. Analysis of cell death by apoptosis in different cell subpopulations



Cells were incubated with BRU25nM, BRU100nM, VEN2.5μM and/or IBR10μM for 24 hours. Differential CLL cells and normal lymphocytes counts were performed using surface markers, CD5 and CD19, and apoptosis was assessed by annexin V staining followed by flow cytometry analysis at 24h. These results are, expressed in percentage (%) normalized to the control. Data are presented as mean ± standard error of mean. The * indicates the cases in which there were statistically significant differences in relation to the control *p<0.01; ***p<0.001.The # indicates the cases in which there were statistically significant differences when compared to BRU 25nM alone, p<0.01. The $ indicates the cases in which there were statistically significant differences when compared to IBR alone, p<0.001. The & indicates the cases in which there were statistically significant differences when compared to VEN alone, p<0.001.

REFERENCES

1. Khodakarami A, Kashani MA, Nazer A, Kheshti AM, Rashidi B, Karpisheh V, et al. Targeted Silencing of NRF2 by rituximab-conjugated nanoparticles increases the sensitivity of chronic lymphoblastic leukemia cells to Cyclophosphamide. Cell Commun Signal. 2023 Aug 1;21(1):188.
2. Sanchez-Lopez E, Ghia EM, Antonucci L, Sharma N, Rassenti LZ, Xu J, et al. NF-κB-p62-NRF2 survival signaling is associated with high ROR1 expression in chronic lymphocytic leukemia. Cell Death Differ. 2020 Jul;27(7):2206–16.
3. Schulz A, Toedt G, Zenz T, Stilgenbauer S, Lichter P, Seiffert M. Inflammatory cytokines and signaling pathways are associated with survival of primary chronic lymphocytic leukemia cells in vitro: a dominant role of CCL2. Haematologica. 2011 Mar 1;96(3):408–16.
4. Wu RP, Hayashi T, Cottam HB, Jin G, Yao S, Wu CCN, et al. Nrf2 responses and the therapeutic selectivity of electrophilic compounds in chronic lymphocytic leukemia. Proc Natl Acad Sci. 2010 Apr 20;107(16):7479–84.
5. Zhang W, Pelicano H, Yin R, Zeng J, Wen T, Ding L, et al. Effective elimination of chronic lymphocytic leukemia cells in the stromal microenvironment by a novel drug combination strategy using redox-mediated mechanisms. Mol Med Rep. 2015 Nov;12(5):7374–88
6. Eichhorst B, Ghia P, Niemann CU, Kater AP, Gregor M, Hallek M, et al. ESMO Clinical Practice Guideline interim update on new targeted therapies in the first-line and at relapse of chronic lymphocytic leukaemia†. Ann Oncol [Internet]. 2024 Jul 3 [cited 2024 Jul 12];0(0). Available from: https://www.annalsofoncology.org/article/S0923-7534(24)00747-6/fulltext
7. Ren D, Villeneuve NF, Jiang T, Wu T, Lau A, Toppin HA, et al. Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. Proc Natl Acad Sci. 2011 Jan 25;108(4):1433–8.
8. Xi W, Zhao C, Wu Z, Ye T, Zhao R, Jiang X, et al. Brusatol's anticancer activity and its molecular mechanism: a research update. J Pharm Pharmacol. 2024 Jul 5;76(7):753–62
9. Lindhagen E, Nygren P, Larsson R. The fluorometric microculture cytotoxicity assay. Nat Protoc. 2008 Aug;3(8):1364–9.
10. Zhang G, Gurtu, Vanessa, Kain ,Steven R., and Yan G. Early Detection of Apoptosis Using a Fluorescent Conjugate of Annexin V. BioTechniques. 1997 Sep 1;23(3):525–31.
11. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol. 2016 Jun 1;17(6):779–90.

DISCLOSURES

The authors declare that they do not have any conflicts of interest.

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