# DEFACTINIB AFFECTS CHRONIC LYMPHOCYTIC LEUKEMIA CELLS SURVIVAL WITH ADDITIVE EFFECTS IN COMBINATION WITH BTK INHIBITORS

N. Mouawad[1], G. Capasso[1], E. Ruggeri[1], A. Visentin[1], F. Severin[1], V. Trimarco[1], A. Tonini[1], S. Pravato[1], M. Facco[1], F. Frezzato[1] and L. Trentin[1].

[1]*Hematology and Clinical Immunology Unit, Department of Medicine, Padua University; Padua, Italy*

abstract ID:1551086

nayla.mouawad@unipd.it

livio.trentin@unipd.it

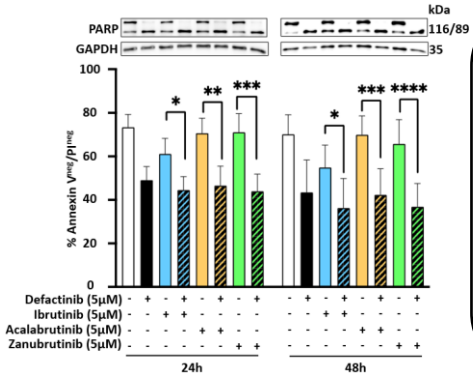
## INTRODUCTION

The emergence of BTK inhibitors has improved the outcome of patients with chronic lymphocytic leukemia (CLL) [1]. The pioneering bruton tyrosine kinase (BTK) inhibitor, ibrutinib, along with the second-generation inhibitors acalabrutinib and zanubrutinib, have displayed remarkable therapeutic efficacy in patients with CLL. Despite the effectiveness of these agents, resistance and severe side effects can arise, resulting in treatment failure, highlighting the need for alternative treatments. Research on molecules involved in the increased survival and drug resistance of leukemic B cells, may unveil novel therapies for a successful outcome in CLL that can be employed clinically alone or in combination with the currently approved BTK inhibitors. Focal adhesion kinase (FAK) is a 125 kDa protein, which upon phosphorylation on tyrosine (Y) 397, is activated and can recruit numerous signalling proteins controlling different processes such as adhesion, migration, apoptosis and proliferation, especially in solid tumors [2]. Consequently, several inhibitors have been developed to target FAK phosphorylation at Y397 or its kinase activity [3]. One of FAK inhibitors is defactinib that has been demonstrated safe and effective in clinical trials for solid tumors. However, limited data are available on the effects of defactinib on CLL. Based on this knowledge, we hypothesized that defactinib may exert a significant effect on the survival of CLL cells, and that its combination with BTK inhibitors may improve their cytotoxic effects, thereby enhancing their *in vitro* activity.

## METHODS

B lymphocytes were isolated from eight CLL patients and then were treated with either 5uM of ibrutinib, acalabrutinib, zanubrutinib alone, or in combination with defactinib at a 1:1 ratio. Parallel experiments were performed where these cells were co-cultured with the human bone marrow stromal HS5 cell line, applying the same treatments. After 24 and 48 hours of treatment, apoptosis was evaluated through two methods: PARP cleavage analysis by western blot and Annexin V/Propidium iodide assay by flow cytometry. The expression level of FAK phosphorylation on Y397 representing its kinase activity, was examined using western blot analysis.

## RESULTS

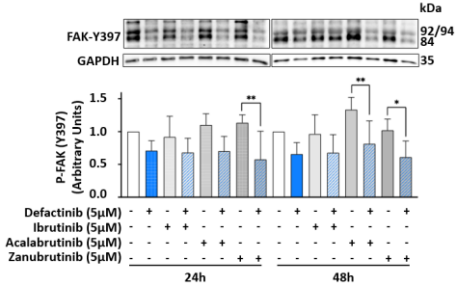


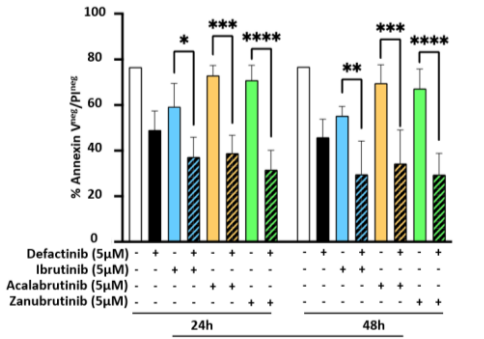

**Defactinib is more powerful than BTK inhibitors in CLL**

Treatment with defactinib alone induces greater apoptosis than BTK inhibitors alone in CLL cells.

**Defactinib boosts BTK inhibitors activity**

When defactinib is combined with BTK inhibitors, an increase in PARP cleavage was observed using western blot analysis (PARP), indicating that defactinib enhances BTK inhibitors activity. This finding was further supported by the Annexin V/Propidium iodide assay, which exhibited a significant reduction in the viability of leukemic B cells when co-treated compared to treatment with either agent alone.




**Selective FAK inhibition enhances CLL apoptosis**

While BTK inhibitors alone didn't affect FAK phosphorylation, defactinib alone or in combination with BTK inhibitors resulted in reduced FAK phosphorylation, as shown by densitometric analysis of western blot (P-FAK Y397), suggesting that the enhanced apoptosis observed in the co-treated conditions is due to the selective inhibition of FAK activation.

**Defactinib disrupts HS5-mediated CLL cell survival**

When CLL B cells were cultured with HS5 stromal cells, known to enhance CLL cell survival *in vivo*, co-treatment of defactinib and BTK inhibitors prevented the protective effect of HS5 cells and instead resulted in a higher levels of apoptosis, as evidenced by Annexin V/Propidium iodide assay. This effect may be attributed to the alteration of FAK in the HS5 cell line upon treatment.

## CONCLUSIONS

In summary, our findings demonstrate that defactinib, by inhibiting FAK activation, exhibits greater power as an apoptotic agent compared to BTK inhibitors. Moreover, defactinib enhances the cytotoxic effects of BTK inhibitors *in vitro* and counteracts the protective effect of HS5 stromal cells on CLL cell survival. These results highlight FAK as a promising target for the development of novel therapeutic approaches in CLL. Furthermore, the combination of FAK inhibitors with BTK inhibitors may enhance their therapeutic efficacy, leading to deeper treatment responses and better outcomes for CLL patients. These findings suggest the potential clinical relevance of targeting FAK and warrant further investigation in clinical settings.

(*) p<0.05 (**) p<0.01 (***) p<0.001

[1] Frustaci et al., Cancers 2023, 15, 1504 [2] Severin & Mouawad et al., Br J Haematol. 2023, 00:1–13 [3] Yoon et al., J Histochem Cytochem. 2015, 63(2):114-128