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Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

# FAK INHIBITION WITH DEFACTINIB ENHANCES BTK INHIBITOR EFFICACY AND OVERCOMES MICROENVIRONMENTAL PROTECTION IN CHRONIC LYMPHOCYTIC LEUKEMIA

**M. Castronuovo[1], A. Beltrame[1], V. Tatangelo[2], L. Lopresti[2], L. Patrussi[2], G. Capasso[1], V. Gramegna[1], P. Fantato[1], V. Trimarco[1], A. Visentin[1], L. Trentin[1], F. Frezzato[1]**

*[1]Hematology Unit, Department of Medicine, University of Padua; [2]Department of Life Sciences, University of Siena.*

**Introduction:** In chronic lymphocytic leukemia (CLL), leukemic cell survival strongly depends on microenvironmental support. Focal adhesion kinase (FAK) integrates signals from surrounding cells and soluble factors, including cytokines, chemokines, and growth factors. Through these interactions, FAK contributes to the establishment of a protective microenvironmental niche that sustains disease persistence. We therefore investigated the impact of FAK inhibition on CLL biology and drug response, focusing on the selective FAK inhibitor defactinib, which is currently under clinical evaluation in solid tumors.

**Methods:** Primary cells from 5 CLL patients were treated with 5 µM defactinib alone or in combination with BTK inhibitors (BTKi; ibrutinib, acalabrutinib, or zanubrutinib). Apoptosis was assessed after 24h by Annexin V/PI staining and PARP cleavage. To evaluate the contribution of the microenvironment, CLL cells were also co-cultured with the HS-5 stromal cell line. To further explore this aspect in a more physiological context, splenocytes obtained from 4 Eµ-TCL1 mouse spleens were cultured with 5 µM defactinib and/or 5 µM ibrutinib, and apoptosis was similarly measured by Annexin V/PI staining. Organotypic spleen cultures from the same model were also analyzed for phospho-FAK (pFAK-Y397) by immunofluorescence following defactinib exposure. Additional assays were performed on leukemic cells derived from ibrutinib-relapsed and heavily pretreated patients (n= 12) to assess whether defactinib retains pro-apoptotic activity in aggressive diseases.

**Results:** Defactinib significantly potentiated BTKi–induced apoptosis in CLL cells. This was showed by increased Annexin V/PI staining ($p<0.0001$) and confirmed by Western blot analysis showing enhanced PARP cleavage together with reduced pFAK-Y397 levels, validating FAK target engagement. The synergistic effect persisted in HS-5 co-cultures, indicating that FAK inhibition overcomes stromal protection. Similarly, in Eµ-TCL1 splenocytes, combined ibrutinib–defactinib treatment showed a trend toward increased apoptosis with respect to single-agent treatments. Organotypic spleen cultures from the same model exhibited a marked reduction in pFAK-Y397 levels following defactinib exposure, confirming its biological activity within the tissue context. Importantly, defactinib also triggered apoptosis ex vivo in leukemic cells derived from ibrutinib-resistant and heavily pretreated patients.

**Conclusions:** Our data suggest that FAK may act as a key regulator of CLL cell survival and therapeutic resistance. Pharmacological FAK inhibition with defactinib enhances BTK inhibitor–induced apoptosis, overcomes microenvironmental protection, and remains active in leukemic cells from therapy-resistant patients. These findings support the therapeutic potential of targeting FAK signaling to disrupt microenvironmental support and improve treatment efficacy in CLL.