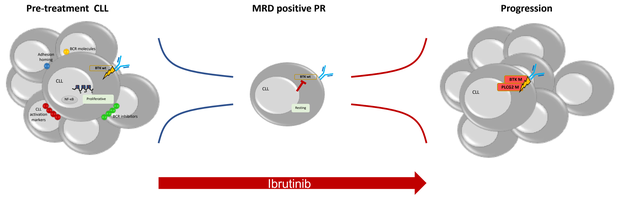
Adaptation of Chronic Lymphocytic Leukemia to Ibrutinib is Mediated by Epigenetic Plasticity of Residual Disease and By-pass Signaling via MAPK

Lodovico Terzi di Bergamo
Institute of Oncology Research
Bellinzona, Switzerland

Background

Chronic Lymphocytic Leukemia (CLL) is characterised by a constitutive activation of the B Cell Receptor (BCR). High-risk CLL patients are treated with ibrutinib, a small molecule that inhibits BTK, downstream the BCR. Most patients only achieve a Minimal Residual Disease (MRD) positive Partial Remission, which is usually kept until disease progression.



Methods

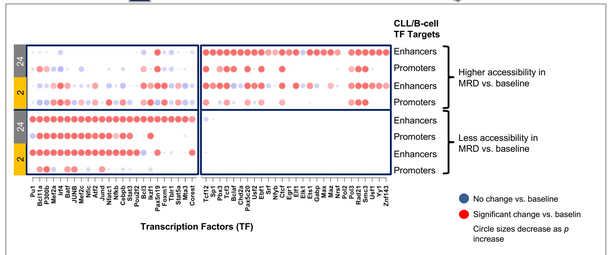
Pre-treatment CLL cells, and under treatment MRD were systematically collected longitudinally from 33 CLL patients who received ibrutinib in the IOSI-EMA001 and IOSI-EMA003 studies. Samples were characterized by multilayer systematic approaches, including high-dimensional single-cell flow cytometry, bulk genomic, transcriptomic and chromatin accessibility profiling and single cell RNA-seq profiling.

Aims

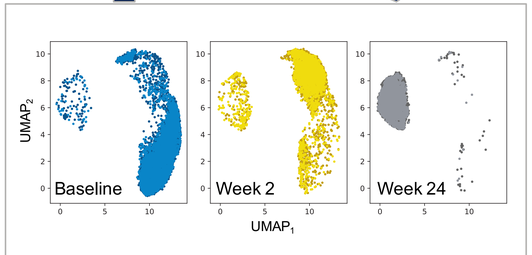
Define the mechanism of adaptation that allows residual CLL cells to survive despite BTK inhibition.

Forestieri G, Loh JW, Singh A, Spina V, Zucchetto A, Condoluci A, Faderl M, Koch R, Bruscaggin A, Pini K, Wu, Piffaretti D, Bittolo T, Tissino E, De Paoli L, Deambrogi C, Frustaci AM, Autore F, Merli M, Scarfo' L, Rasi S, Passweg J, Moia R, Martines C, Ghia P, Cavalli F, Zucca E, Gerber B, Gillessen S, Stüssi G, Montillo M, Passamonti F, Gregor M, Laurenti L, Tedeschi A, Gaidano G, Efremov D, Gattei V, Khiabanian H, Rossi D

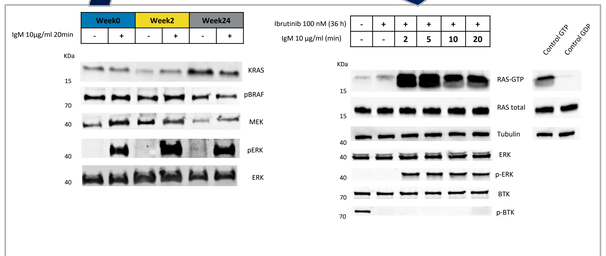
Results



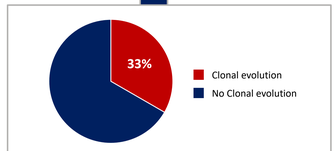
(B) ATAC-seq. Decreased accessibility regions are enriched in binding sites of TF emanating from the BCR signalosome (NF-kB, p38, JNK) **Increased accessibility** regions are enriched in binding sites of TF downstream ERK signaling and controlling early B cell development



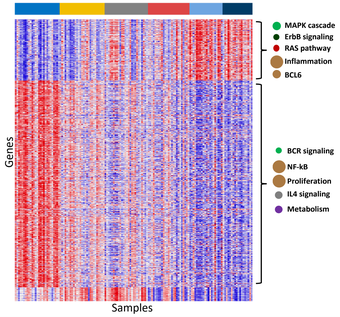
(D) scRNA-seq. Single-cell transcriptomes revealed a distinct post-treatment cell population driven by MAPK activity which functionally pre-existed in a fraction of CLL cells of the baseline samples before ibrutinib start.



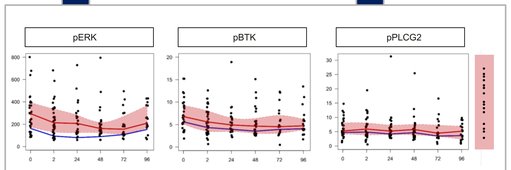
(F) Functional validation on primary samples by western blotting shows that RAS/ERK can be activated by BCR stimulation irrespective of ibrutinib treatment in ex vivo experiments. Pharmacological inhibition of MEK (trametinib) and ERK (ulixertinib) synergized with ibrutinib leading to decreased cell viability (not shown).



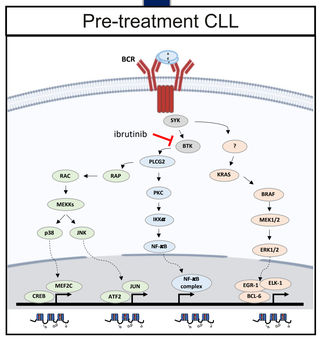
(A) DNA-seq. MRD survival is not driven by genetic clonal evolution

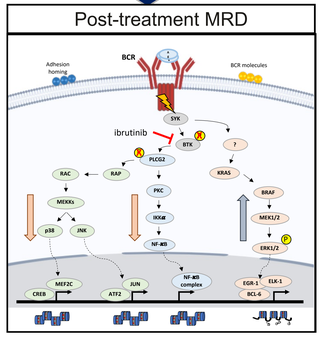


(C) Bulk RNA-seq. Most of the differentially expressed genes between pre- and post-treatment samples were downregulated in MRD, while only a fraction was upregulated. IL4, NF-kB, metabolism and proliferation signatures were downregulated in MRD, while MAPK and RAS signatures were upregulated.



(E) Phosphoflow cytometry. pBTK and pPLCG2 were not active in MRD due to ibrutinib. Upon crosslinking of the BCR with anti-IgM, the density of expression of the pERK increased in MRD cells at levels that were similar to those measured in the pre-treatment samples. However, this was not observed after stimulation of TLR9 or CD40.





Conclusions

MRD under ibrutinib adapts its phenotype, mainly in an epigenetic way to maintain functional competence of BCR signaling through the MAPK pathway. ERK signaling remains active despite BTK inhibition and leads to epigenetic reprogramming. MAPK pathway turned to be a vulnerability of MRD persisting under ibrutinib, leading to cell death after both MEK and ERK inhibition.