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POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Multi-Omics Exploration of Adaptive Mechanism to BTK Inhibition By Ibrutinib in CLL Identified TMBIM6/BI-1 As a Poor Prognosis Variable and Potential Therapeutic Target

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Introduction: Ibrutinib is currently part of the therapeutic armamentarium employed in patients with CLL. Despite being an active drug, its usage is not devoid of adverse effects and resistances leading to discontinuation. In addition, current evidence suggests that ibrutinib improves the T cell disfunction associated with CLL progression. The objective of our study was to longitudinally assess the T cell immune profile of ibrutinib-treated CLL patients, and to identify potential adaptive mechanisms to BTK inhibition in B cells in the first 12 months of continuous treatment.
Methods: Blood samples from 28 patients from GELLC7 trial (NCT03280160, ibrutinib followed by ofatumumab consolidation) were collected before treatment (BT), and 1, 3, 6 and 12 months on treatment (at least two timepoints). Peripheral blood mononuclear cells and plasma were isolated and stored to perform multi-omics analyses including flow cytometry, RNA sequencing, cytokine determination, migration assays and targeted DNA sequencing.
Results: T cell immunophenotyping revealed a decrease of CD4+ and CD8+ T cells expressing PD1 and CD244 and a reduction of regulatory T cells (Tregs) and T follicular helper (Tfh) cells after ibrutinib treatment. In addition, bulk-RNAseq of purified T cells revealed 170 differentially expressed genes (DEG) after 6 months of ibrutinib treatment. These genes participate in pathways related to cell stimulation and regulation of cell activation. Gene set enrichment analysis (GSEA) showed a downregulation of oxidative metabolism, proliferation, and activation pathways, indicating a reduction of the CLL inflammatory burden. Furthermore, there was a significant reduction of several inflammatory/migration chemokines in plasma at 6 months (CCL2/3/4, CXCL10, CXCL12, CXCL13, CCL19).
In CLL cells, a decreased expression of adhesion (CD44/CD62L), immunosuppression- (CD200/BTLA), and migration-related markers (CXCR5/CCR7) at 6 months of ibrutinib treatment was observed. However, CXCR4 expression increased upon ibrutinib treatment, and the proliferative CXCR4dimCD5br population was reduced.
We investigated CXCR4-mediated migration as a potential mechanism of CLL cell adaptation to ibrutinib in 14 patients at BT and 6 months of BTK inhibition. Reduced CXCR4-migratory capacity was observed, but some cells retained migration under BTK inhibition. Targeted-DNAseq and bulk-RNAseq were performed at BT and 6 months of ibrutinib to characterize CLL cells with retained migratory capacity. There was an alteration of the subclonal composition of CLL cells that retained migratory capacity under BTK inhibition in 7/12 patients with a recurrent enrichment of subclones with mutations in *ATM* and *NFKBIE* gene. *BTK* p.Cys481Ser mutation was acquired in one patient after 6 months of treatment with ibrutinib, while no *PLCG2* mutations were detected. GSEA showed an upregulation of mTORC1 and Myc pathways in cells with retained migration.

Among the DEG, *TMBIM6* was the most significantly upregulated gene. *TMBIM6* gene is associated with apoptosis, mTOR-Akt axis and poor prognosis in solid tumors. We evaluated the prognostic impact of *TMBIM6* expression using public data from the CLL-map portal (Knisbacher et al. 2022 Nat Genet). *TMBIM6* high expression was associated with poor overall survival in both univariate and multivariate analyses including age, gender, *IGHV* mutation status (Figure 1) and recurrent genetic alterations. When TMBIM6 was targeted with BIA, a TMBIM6 antagonist (Kim et al. 2020 Nat Commun), its effect on CXCR4-mediated migration was limited but it induced CLL cell apoptosis in suspension and co-cultured CLL cells (Figure 2). Moreover, BIA addition to ibrutinib treatment induced synergistic apoptosis. Collectively, these results indicate that TMBIM6 may be a potential novel target for CLL and that combination with ibrutinib could be a valid approach for time-limited therapy.
Conclusions: Ibrutinib in T cells reduced the expression of exhaustion markers, Tregs and Tfh cells. In CLL cells, we observed a downregulation of markers related to adhesion, immunosuppression and migration, but an overexpression of *TMBIM6* in CLL cells that retained migrative capacity towards CXCL12 under ibrutinib. Finally, we identified *TMBIM6* expression as an independent poor prognostic factor and a potential novel target for CLL.

Disclosures Cabirta Touzón: *AbbVie:* Other; *BeiGene:* Honoraria; *AstraZeneca:* Honoraria; *Janssen:* Honoraria. **Ferrà:** *Janssen:* Consultancy; *Roche:* Consultancy; *Gilead:* Consultancy; *Takeda:* Consultancy; *Abbvie:* Consultancy; *Janssen:* Other: medical meetings funding; *Roche:* Other: medical meetings funding; *Gilead:* Other: medical meetings funding; *Abbvie:* Other: medical meetings funding. **Alcoceba:** *Janssen, AstraZeneca:* Honoraria, Other: Travel expenses. **Terol:** *Janssen:* Membership on an entity's Board of Directors or advisory committees, Other: Travel, Research Funding; *Abbvie:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Other: Travel; *Gilead:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Other: Travel, Research Funding; *BMS:* Consultancy; *Roche:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Other: Travel; *Takeda:* Membership on an entity's Board of Directors or advisory committees, Other: Travel; *Hospital Clínico Valencia:* Current Employment. **Bosch:** *Novartis:* Consultancy, Honoraria; *AbbVie:* Consultancy, Honoraria; *Janssen:* Consultancy, Honoraria; *Gilead:* Consultancy, Honoraria; *Mundipharma:* Consultancy, Honoraria; *Lilly:* Consultancy; *Roche:* Honoraria; *BeiGene:* Consultancy; *AstraZeneca:* Consultancy, Honoraria; *Takeda:* Consultancy, Honoraria; *Karyospharm:* Other; *Celgene:* Consultancy, Honoraria; *Roche:* Consultancy, Honoraria. **Abrisqueta:** *Beigene:* Consultancy; *BMS:* Consultancy, Honoraria, Speakers Bureau; *Roche:* Consultancy, Honoraria, Speakers Bureau; *Abbvie:* Consultancy, Honoraria, Speakers Bureau; *Janssen:* Consultancy, Honoraria, Speakers Bureau; *Incyte:* Honoraria, Speakers Bureau; *Astrazeneca:* Consultancy, Honoraria, Speakers Bureau.

Figure 1

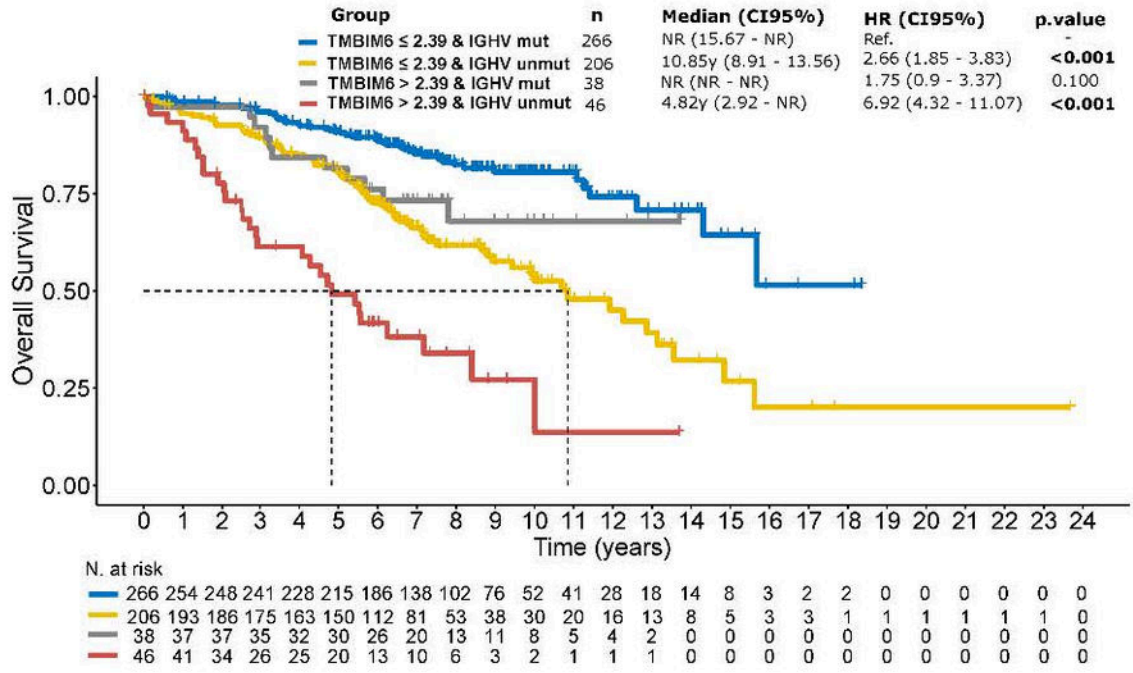

Figure 2

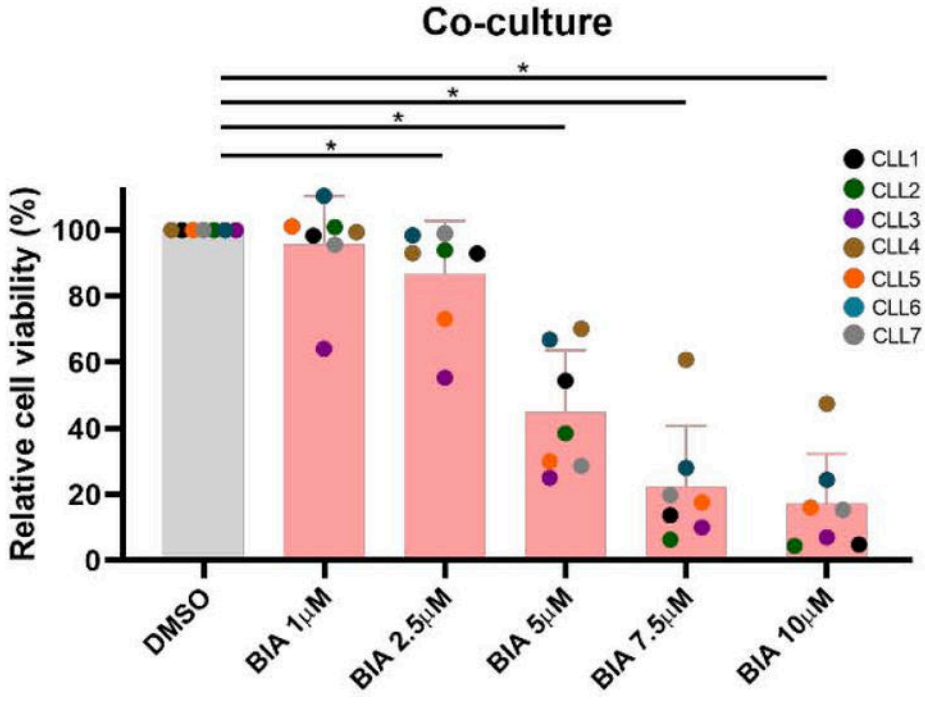

Figure 1

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