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The S100A9/EMMPRIN axis is involved in chronic lymphocytic leukemia progression and offers novel therapeutic opportunities

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OBJECTIVES

We recently identified the S100A9/EMMPRIN pathway as a novel driver of CLL progression. Here, we focus on a specific branch of this pathway (S100A9 → EMMPRIN → metalloproteinases axis) to evaluate its role in the context of BTK inhibitor therapies.

CONCLUSIONS

- ***S100A9/EMMPRIN pathway induces molecules associated with CLL homing during disease progression***
- ***S100A9/EMMPRIN/MMPs axis is principally activated in the proliferating CLL fraction***
- ***BTK Inhibitors Block S100A9/EMMPRIN/MMPs, down-modulating molecules involved In the homing of CLL cells in treatment-responsive patients***

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INTRODUCTION

Chronic Lymphocytic Leukemia (CLL) is marked by clonal B cell expansion in peripheral blood (PB), bone marrow (BM), and secondary lymphoid organs (SLO). Poor prognosis is associated with increased proliferation of CLL cells, driven primarily by interactions with the tumor microenvironment (TME), making these interactions an important therapeutic target.

Our lab previously identified S100A9, a pro-inflammatory protein enriched in serum-derived exosomes from poor-prognosis patients. These exosomes activate NF-κB in leukemic cells through S100A9/EMMPRIN pathway (Prieto, Seija and Sotelo et al. 2017). *Extracellular Matrix Metalloproteinase Inducer* (EMMPRIN), the receptor for dimeric S100A9 triggers NF-κB, PI3K/AKT, and MAPK/ERK pathways in CLL cells, suggesting a novel progression-related signaling axis in CLL (Uriepero-Palma, Márquez, and Payque et al. 2025). Given that EMMPRIN also induces metalloproteinases (MMP2, MMP9), linked to CLL infiltration and disease progression (Redondo-Muñoz et al., 2021). We therefore asked whether inhibition of the S100A9/EMMPRIN axis affects CLL cell homing to proliferative centers (PCs). Since BTK inhibitors (BTKi) disrupt TME interactions and regulate the egress and/or entry of CLL cells between PCs and peripheral blood (Ponader et al., 2012; Chen et al., 2016), we hypothesize that S100A9/EMMPRIN activation drives MMP expression required for re-entry into PCs, while BTKi block this pathway, reducing CLL proliferation and MMP2/9 expression, ultimately preventing homing.

This work provides further insight into the complex molecular mechanisms through which BTKi exert their effects on CLL cells, describing an additional mode of action that helps explain their therapeutic success in CLL.

METHODS

CLL samples were obtained with informed consent and Ethics Committee approval. PBMCs before and after treatment were collected and stored in the GURU-LLC Biobank (project GURU-01). *In-vitro* activation experiments with S100A9 and inhibition by BTKi were performed using PBMCs from untreated CLL patients. *Ex-vivo* studies with PBMC from responder ibrutinib treated patients were conducted.

RESULTS

S100A9 stimulation activates the EMMPRIN/MMPs axis in CLL cells, increasing MMP2, MMP9, CCL3, and CCL4 mRNA, all key molecules involved in migration and invasion. This axis also appears to be regulated by CD40L/IL-4 signaling through upregulation of the transcription factor (TF) SP1, previously described as a major regulator of EMMPRIN transcription. Overexpression of EMMPRIN further enhances MMP9 expression in CLL cells.

Interestingly, the SP1 → EMMPRIN → MMP9 pathway was down-modulated by in vitro treatment with two different BTKi (Ibrutinib, 1 μM; BTK degrader NX-0492, 2 nM; 24 h). These findings were confirmed ex vivo in CLL cells from Ibrutinib-treated patients, supporting that BTKi counteract the pro-migratory program.

Based on previous reports describing BTKi effects on proliferative CLL fractions (Morande and Sivina et al., 2019), we investigated the role of Ibrutinib on the SP1 → EMMPRIN → MMP9 axis in proliferative fractions (PF; IgM+/IgG+ or CXCR4^{low}/CD5^{high}). Our results showed that this axis is markedly increased in proliferative and dividing fractions compared with their resting counterparts. Notably, Ibrutinib specifically reduced SP1 → EMMPRIN → MMP9 expression in actively proliferating cells, potentially impairing leukemic cell homing and promoting tumor cell death.

1. Activation of S100A9/EMMPRIN axis increases molecules associated with CLL migration and invasion, is dependent of CD40/IL-4 signaling and downmodulated by BTKi

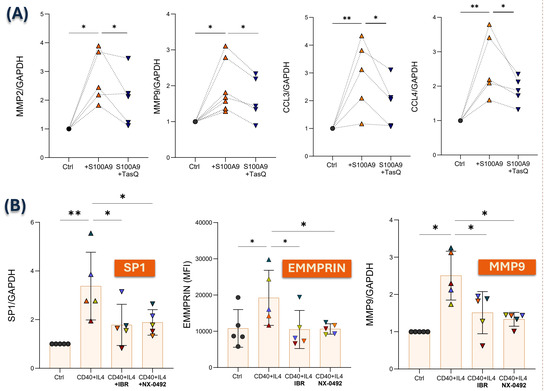

Fig. 1A. Metalloproteinases MMP2 and MMP9, and the chemokines CCL3 and CCL4, associated with tumor migration and homing after stimulation with rhS100A9, with or without an S100A9 inhibitor (Tasq). Cells were stimulated with rhS100A9 (10 μg/mL) in the absence or presence of Tasq (10 μM) for 48 hours in RPMI-1640 medium supplemented with 10% FBS. Total RNA was then extracted and the expression of MMP2, MMP9, CCL3, and CCL4 was assessed by qPCR. **Fig. 1B. BTK inhibitor treatment reduces SP1, EMMPRIN, and MMP9 expression in vitro.** Primary CLL cells were stimulated with CD40L + IL-4 for 5 days and treated with either ibrutinib (1 μM) or BTK degrader NX-0492 (2 nM) for 24 h prior to the end of stimulation, in RPMI-1640 medium supplemented with 10% FBS. Total RNA was extracted to assess SP1 and MMP9 expression by qPCR, while EMMPRIN levels were determined by flow cytometry. Data were analyzed using a paired t-test,and ONE-way ANOVA, *p < 0.05 and **p < 0.005.

2. What is the effect of BTKi on SP1, EMMPRIN, MMP-9 and CCL3/4 in CLL cells of treated patients?

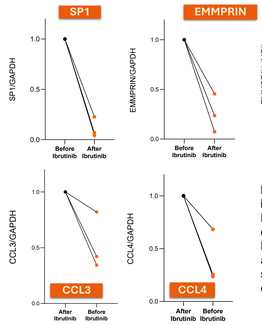

Fig. 3. Ex vivo analysis of CLL cells from ibrutinib-treated patients. SP1, EMMPRIN, MMP9, CCL3, and CCL4 mRNA levels were quantified by qPCR before and after treatment. In responder patients, CLL cells were collected after 3 months of ibrutinib therapy. EMMPRIN surface expression was also assessed by flow cytometry.

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4. SP1→EMMPRIN→MMP9 expression are increased in the PF ongoing CSR compared with RF

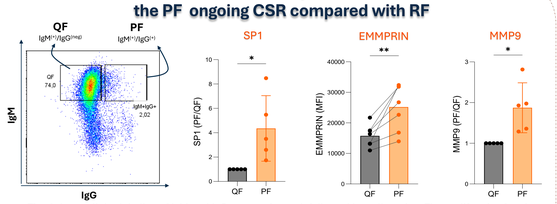
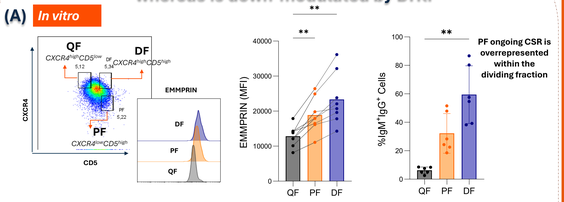


Fig. 4. Intracellular labeling of IgM and IgG was performed, followed by cell sorting. The proliferative fraction (PF) was defined as CD19+/IgM+/IgG+ cells, and the quiescent fraction (QF) as CD19+/IgM+/IgG− cells. RNA was extracted from each subset to assess SP1 and MMP9 expression. EMMPRIN surface expression was measured by flow cytometry as MFI in each fraction. Data were analyzed using a paired t-test, with significance indicated as *p < 0.05 and **p < 0.005.

5. EMMPRIN is up-regulated in proliferative (CXCR4^{low}CD5^{high}) and dividing fractions (CXCR4^{low}CD5^{high}) whereas is down-modulated by BTKi



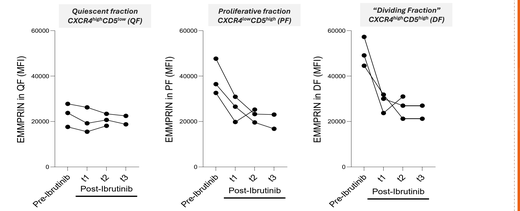
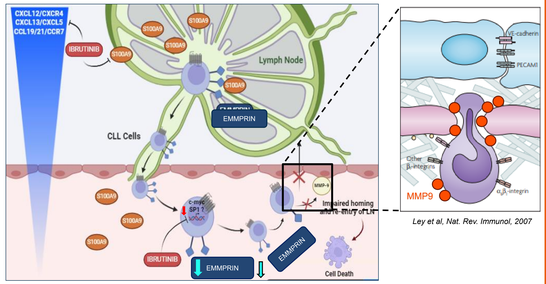


Fig. 5. EMMPRIN expression in dividing, proliferative, and quiescent fractions. A) The PF was defined as CD19^{+}/CXCR4^{low}/CD5^{high}, the dividing fraction (DF) as CD19^{+}/ CXCR4^{high}/CD5^{high} as reported in 6. and quiescent fraction (QF) as CD19^{+}/CD5^{low}/CXCR4^{high}. EMMPRIN expression in each subset was assessed by FC in primary treatment-naïve CLL cells. Paired t-test **p < 0.005. **B)** The percentage of IgM^{+}/IgG^{+} cells within each subset was measured as described in 7. Ongoing CSR within the DF is overrepresented. **C)** All subsets were analyzed in patients before and after ibrutinib treatment (t1 = 1 week; t2 = 1 month; t3 = 3 months).

Proposed model: Ibrutinib disrupts the S100A9/EMMPRIN axis and collaborate to impair tumor niche homing



Ibrutinib treatment reduces the expression of SP1/EMMPRIN/MMP9. As a result, leukemic cells may lose the ability to re-enter proliferative niches, impairing extracellular matrix degradation. This could lead to the absence of survival and proliferative signals, ultimately promoting leukemic cell death.