# Prognostic significance of TET and IDH mRNA expression in chronic lymphocytic leukemia

M. Van Damme*, E. Crompot, N. Meuleman, P. Mineur, D. Bron, L. Lagneaux and B. Stamatopoulos

**Laboratory of Clinical Cell Therapy (LTCC) - ULB - Jules Bordet Institute, Brussels, Belgium *mvdamme@ulb.ac.be**

## *Background*

Epigenetics plays a crucial role in cancer physiopathology. **DNA hydroxymethylation** is catalyzed from methylated DNA by Ten Eleven Translocation (**TET**) enzymes requiring co-factors produced by Isocitrate Dehydrogenase (**IDH**) proteins. This modification could be a step in the **demethylation process** and could thus have an impact on gene expression. TET2 mutations have already been reported in several leukemias. However, little is known about hydroxymethylation in **Chronic Lymphocytic Leukemia** (CLL).

## *Methods*

Expression of TET1,2,3, IDH1 and 2 mRNA was assessed by **qPCR** on purified leukemic B-cells from a cohort of **214 CLL patients** with a median follow-up of 75 (6-380) months and compared with those of purified peripheral normal B-cells. The influence of **mesenchymal stromal cells** contact on TET enzyme expression, was **investigated in culture of CLL cells (n=10) with presence or absence of bone marrow mesenchymal** stromal cells (BMSC). DNA hydroxymethylation has been measured on CLL B-cells by using MethylFlash Hydroxymethylated DNA quantification Kit (Epigentek).

## *Results*

**TET 1, 3 and IDH2 were underexpressed** in leukemic B-cells compared with healthy volunteer B-cells (P=0.0221, 0.0013, <0.0001 respectively) while **IDH1 was overexpressed** (P=0.0037). Expression of TET2 was not statistically different between both groups (Figure 1).

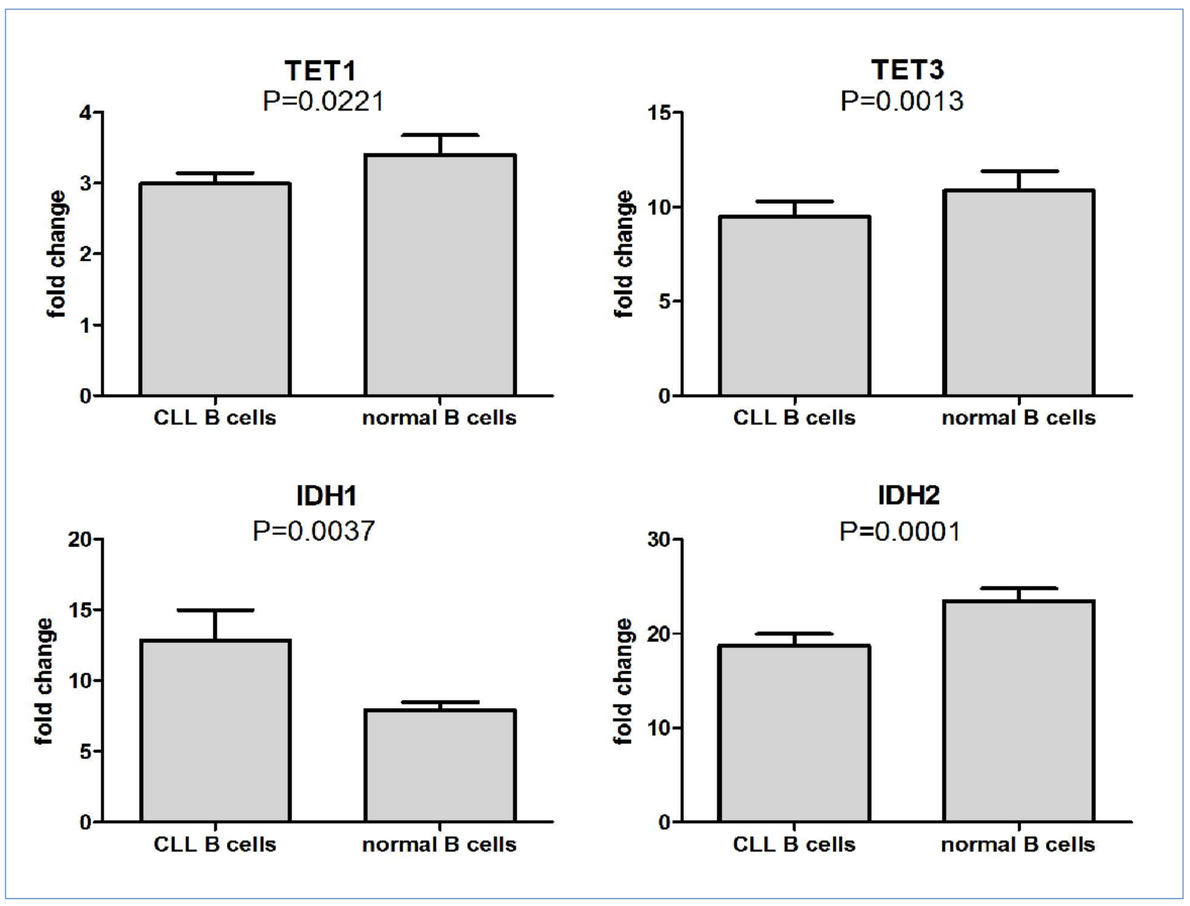


**Figure 1** : mRNA expression of TET1, TET3, IDH1 and IDH2 in CLL and normal B cells (mean and SD).

When we stratified patients according to low and high expression (cut-offs calculated by recursive partitioning) , **TET2 and IDH1 significantly predicted treatment-free survival (TFS)**: patients with high TET2/IDH expression had a median TFS of 110 months while patients with low expression presented a median TFS of 78 months (P=0.0071/0.0123) (Figure 2).

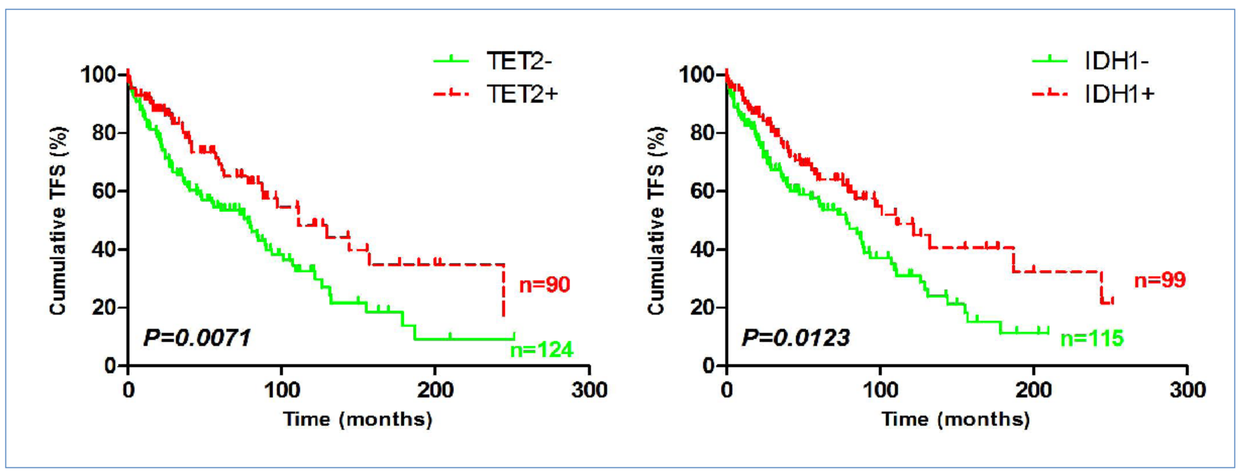


**Figure 2** : TFS Kaplan-Meier curves for CLL patients according to their TET2 and IDH1 expression.

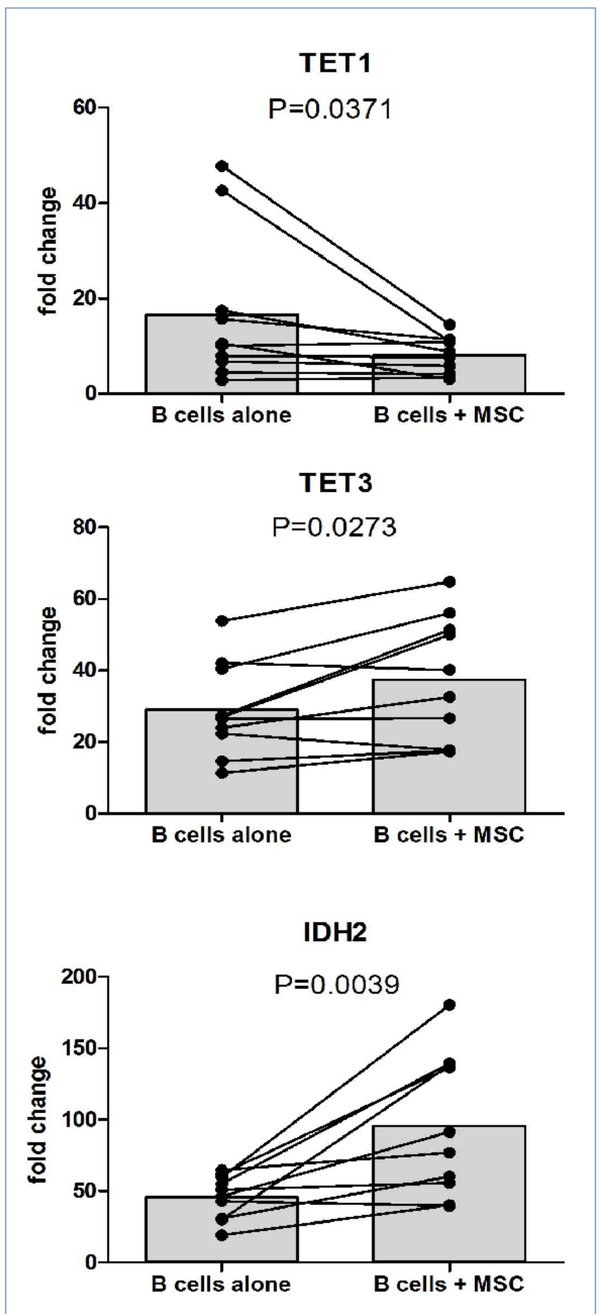


**Figure 3**: TET1, TET3 and IDH2 expression in B CLL cells with and without contact with MSC.

Finally, we observed a **decreased TET1 expression** (P=0.0371) and an **increased TET3** (P=0.0273) and **IDH2 expression** (P=0.0039) in CLL-cells after **co-culture with BMSC** (Figure 3).

CLL is characterized by an heterogeneous clinical outcome that can be predicted by several prognostic factors. Of these, ZAP70 expression is associated to a poor prognosis. Further analysis in 10 CLL patients shows that **ZAP70+ patients present higher hydroxymethylated DNA than ZAP70- patients** (P= 0.0325) (Figure 4).

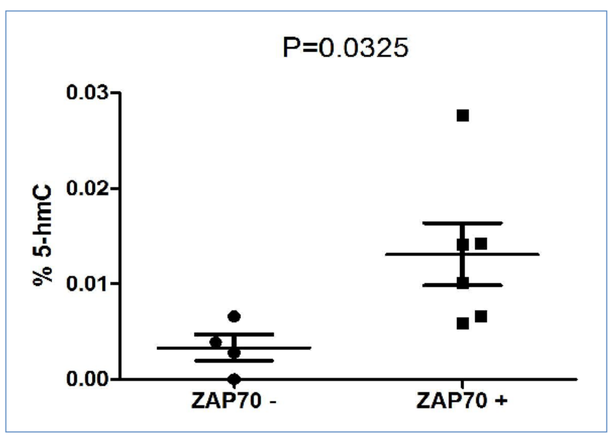


**Figure 4** : DNA hydroxymethylation status in ZAP70- and ZAP70+ patiens (mean and SD).

## *Conclusion*

This is the first report demonstrating that DNA hydroxymethylation enzymes are deregulated in CLL compared to normal B cells: 1) **TET2 and IDH1 overexpression have a good prognostic value**; 2) **TET1, TET3 and IDH2 expression are modulated by microenvironment interactions**, resulting in CLL cell survival. Surprisingly, **global DNA hydroxymethylation is higher in patients with poor prognosis based on ZAP70 status**. Our observations suggest that DNA hydroxymethylation plays a major role in CLL physiopathology and support the potential therapeutic benefit of agents targeting epigenetics in CLL patients. Further experiences with RNA interference will be performed to determine the involvement of these enzymes in biological functions and physiopathology.

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CLL
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