EZH2 TARGETING INDUCES CD38 UPREGULATION AND RESPONSE TO ANTI-CD38 ANTIBODIES IN MULTIPLE MYELOMA

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ABSTRACT

Multiple myeloma (MM) is the second most common hematological malignancy characterized by the accumulation of tumor plasma cells within the bone marrow. MM is characterized by high molecular and clinical heterogeneity. During the last 10 years, new therapeutic classes including **targeted immunotherapies** significantly improved the overall survival of MM patients, but **drug resistance** and relapse remain major challenges. Resistance to CD38 targeted immunotherapies has been associated with marked reduction of CD38 expression via exocytosis, endocytosis, degradation of the antigen-antibody complex and trogocytosis.

Here, we demonstrated that treatment of MM cells with **EZH2 inhibitor** leads to a significant **upregulation of membrane CD38 expression** in cell lines and primary MM cells from patients. Interestingly, CD38 re-expression was linked to an improvement of **Daratumumab** and **Isatuximab** ADCC efficiency. Overall EZH2 targeting may be of therapeutic interest to overcome resistance to anti-CD38 targeted immunotherapies in Multiple Myeloma.

1) Negative correlation between EZH2 and CD38 expression

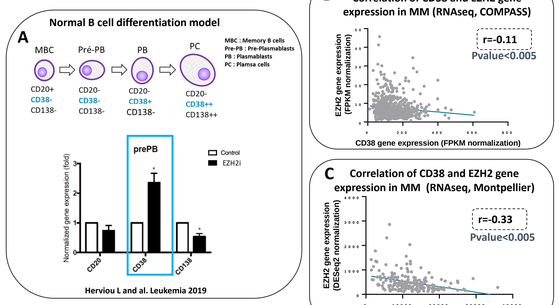
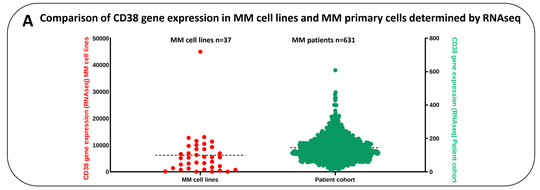


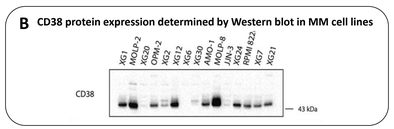
Figure 1: Identification of a link between EZH2 (Enhancer of Zeste homolog 2) expression and CD38 expression in primary cells

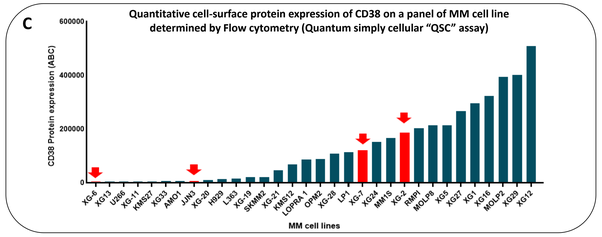
A) In our *in vitro* B to plasma cell differentiation model a link between CD38 and EZH2 expression was demonstrated with a transcriptional control of CD38 expression involving polycomb PRC2 complex (*P<0.05). **B) and C)** In 2 independent cohorts of MM patients (Compass n=631 and Montpellier n= 198) a significative negative correlation (r) between CD38 and EZH2 gene expression was identified (P<0.05).

We hypothesized that PRC2 targeting (with EZH2 inhibitor) could induces CD38 re-expression in Multiple Myeloma.

2) Long term EZH2 inhibition increases CD38 protein expression in 3 MM cell lines







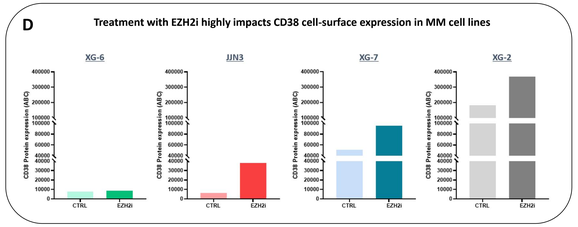
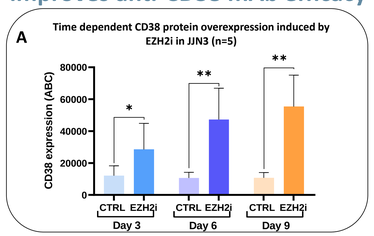
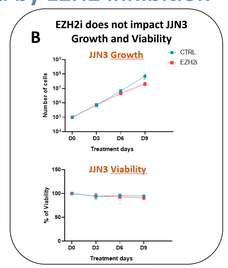


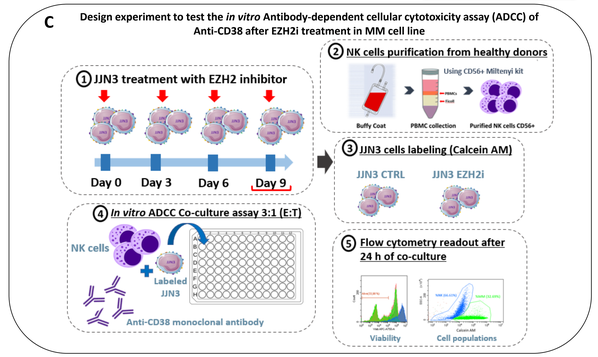
Figure 2: CD38 membrane protein characterization and overexpression on MM cell lines using EZH2 inhibitor

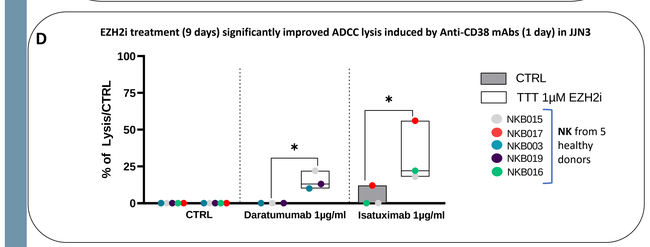
A) Heterogeneous CD38 gene expression in our unique panel of MM cell lines (n=40) as observed in MM patient cohort (n=631) determined by transcriptomic data (RNAseq). **B)** Heterogeneous CD38 protein expression determined by Western blot on MM cell lines (n=15). **C)** CD38 membrane expression assessed by flow cytometry (using QSC kit to calculate the Antibody Binding Capacity (ABC)) on a unique panel of human myeloma cell lines (n=32) representative of MM heterogeneity. **D)** Long term EZH2 inhibitor treatment (1µM of Tazemetostat for 12 days) induces an increase in cell-surface CD38 expression on 3 MM cell lines (JJN3, XG-7 and XG-2).

3) CD38 protein expression induced by EZH2 inhibition improves anti-CD38 mAb efficacy









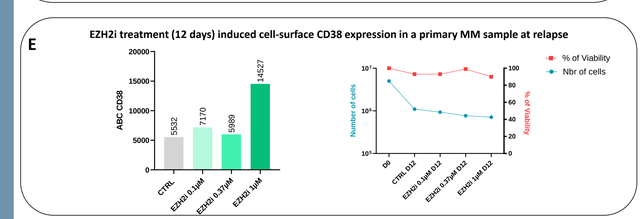


Figure 3: EZH2 inhibitor treatment improves Anti-CD38 monoclonal antibodies efficacy in MM models.

A) EZH2i (1µM of Tazemztostat) increases CD38 expression in JJN3 cell line at day 3, Day 6 and Day 9 (n=5).

B) Tazemetostat treatment (1µM EZH2i) does not impact JJN3 growth and viability. **C)** Design experiment to test the in vitro Antibody-dependent cellular cytotoxicity assay (ADCC) of Anti-CD38 after EZH2i treatment in MM cell line **D)** 9 days of 1µM EZH2 inhibitor treatment increases JJN3 lysis induced by Daratumumab (1µg/ml) and Isatuximab (1µg/ml) two monoclonal antibodies targeting CD38 (n=3) (Paired T-test, Pvalue< 0.05). **E)** 12 days of Tazemetostat treatment (1µM EZH2i) induces CD38 re-expression in MM primary sample collected at relapse after Daratumumab treatment. No effects on the viability and total number of cells were observed after EZH2i treatment.

4) Epigenetic landscape of CD38 promoter shows a correlation between activation H3K4me3, inhibition H3K27me3 Histone marks, and CD38 gene expression in MM cell lines

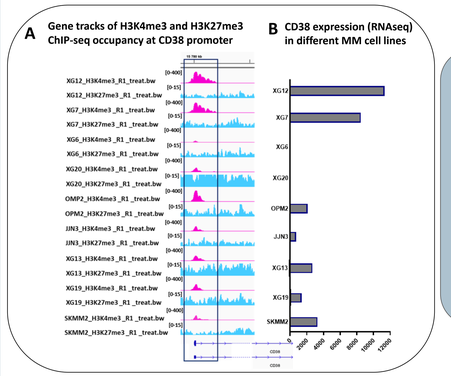
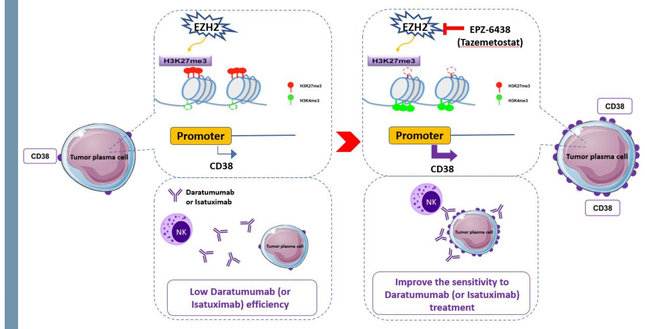


Figure 4: Epigenetic landscape correlates activation and inhibition marks with CD38 expression in MM cell lines

A) Histone marks on lysine residues (activation mark (H3K4me3) and inhibitor mark (H3K27me3)) in CD38 promoter were determined by ChIPseq in 9 MM cell lines. The lack or presence of H3K4me3 histone mark in CD38 promoter could explain the EZH2i effect on CD38 expression observed in Figure 2.

B) CD38 expression in 9 MM cell lines determined by RNAseq analysis.

CONCLUSIONS



- Negative correlation between EZH2 and CD38 gene expression is observed
- Long term EZH2 inhibitor treatment increased cell-surface CD38 expression in MM cell lines and in primary MM sample
- EZH2 inhibitor treatment improves the MM cell lines sensitivity to Daratumumab and Isatuximab
- EZH2 inhibitor response and CD38 expression are associated to CD38 promoter epigenetic landscape

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