BET Bromodomain inhibition: a new strategy for immunotherapy in MPN

Niloofar Amirian[1], Anya Squires[1], Lauretta Azanabor[1], Claire L Walker[1], Matthew J Simmonds[1]*& Ciro R Rinaldi[1,2]*
1-School of Natural Sciences, College of Health & Science, University of Lincoln, UK
2- United Lincolnshire Hospitals NHS Trust
* = Joint Senior authors
Key words: MPN, BET inhibitors, CD47, CALR

Current MPN Challenges

Myeloproliferative neoplasms (MPN), are a group of blood cancers characterized by excessive blood cells production & a significant risk of transformation into acute myeloid leukaemia (AML).

Existing therapies, such as Hydroxyurea and Ruxolitinib, mainly provide partial symptom control, with nearly half of patients showing no response. This underscores an urgent need for improved treatments, particularly those targeting immune evasion pathways like CD47/CALR.

BET inhibitors

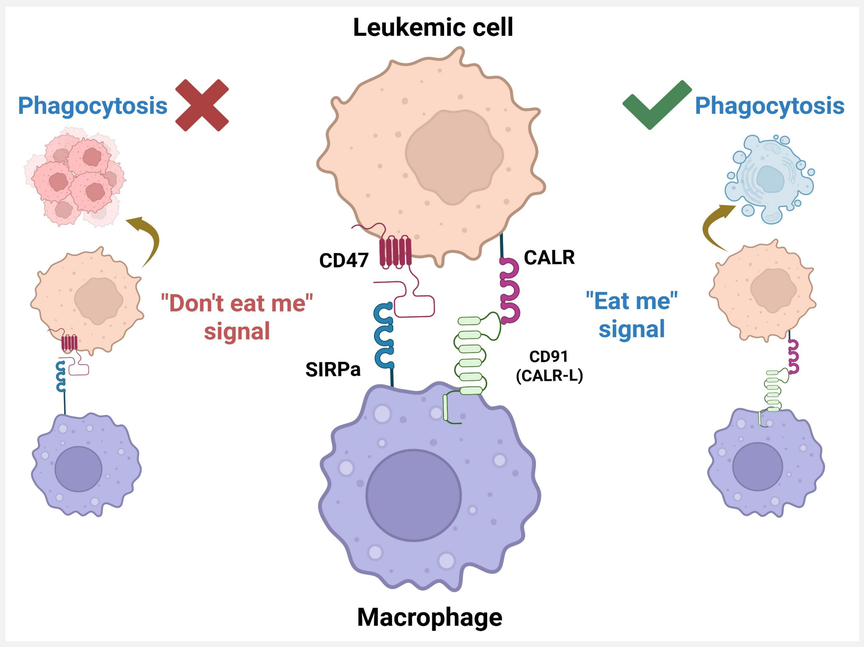
The bromodomain and extraterminal (BET) family regulates diverse cellular functions.

Within this group, the BET family, especially BRD4, supports cancer cell survival by activating RNA polymerase II & driving oncogene expression, including MYC, a key player in malignancies.

Consequently, BET inhibition has emerged as a promising therapeutic strategy in cancer treatment.

Targeting CD47-CALR

On cancer cells, CD47 serves as a “don’t eat me” signal, preventing macrophage-mediated phagocytosis. Conversely, calreticulin (CALR) acts as an “eat me” signal, facilitating the phagocytic elimination of malignant cells.



Figures created at Biorender.com.

A balance between CALR & CD47 signals is required to trigger the macrophage-mediated phagocytosis cascade, promoting the removal of leukemic cells from the bone marrow & enhancing anti-tumour immunity.

We have shown that levels of CD47 alter in MPN as disease progresses & can impact successful treatment outcome.

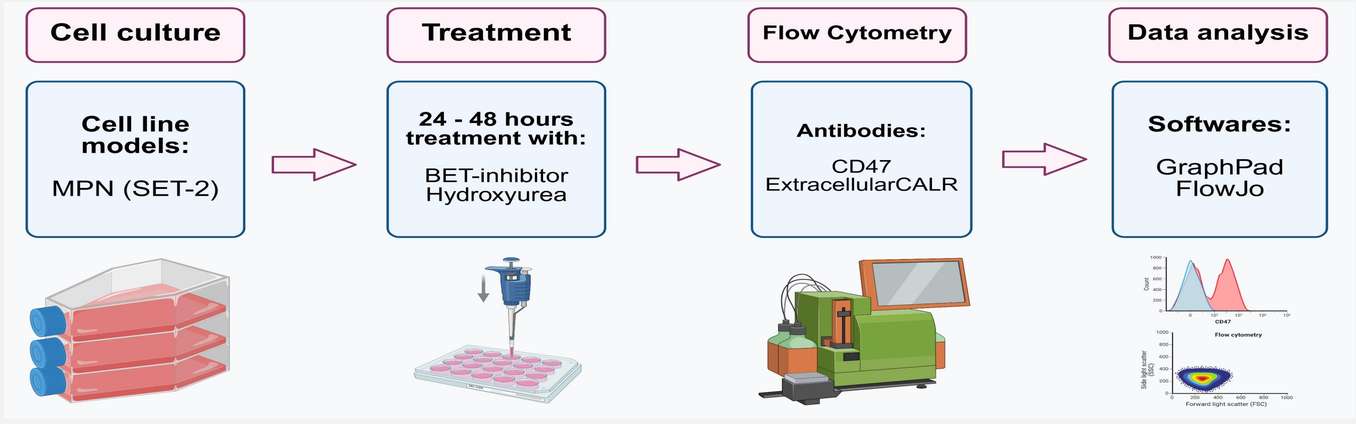
References

1- Weiskopf K. European Journal of cancer. 2017;76:100-9.
2- Chao MP.; Ash A. Alizadeh; Chad Tang. Cancer Research. 2011;71(4):1374-84.
3- Cencini E.; Alberto Fabbri; Anna Sicuranza. Cancers. 2021;13(14):3597.
4- Boaman, K, Walker CL, Simmonds MJ & Rinaldi CR, Haematologia 2025, In Press

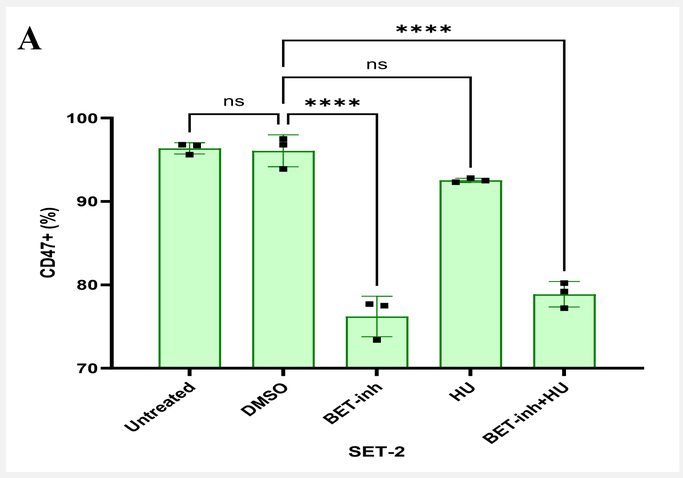
Aim

To investigate the impact of BET inhibition on the CD47-CALR signalling pathway, both as a monotherapy approach & in combination with Hydroxyurea, a standard therapy for MPN.

Methods



Results



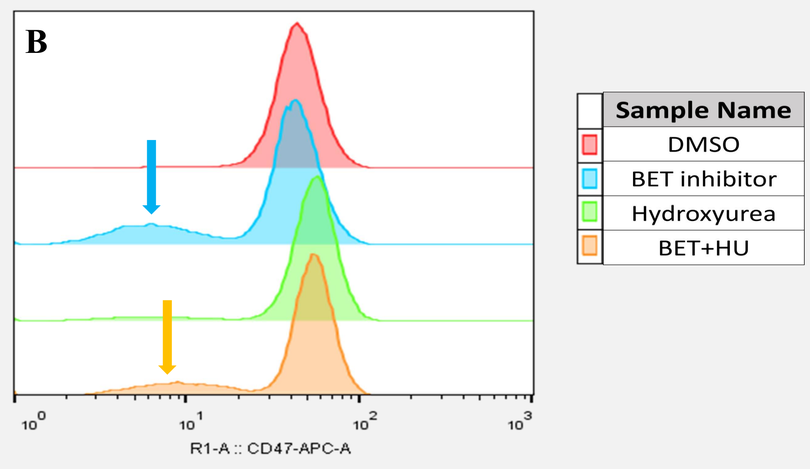
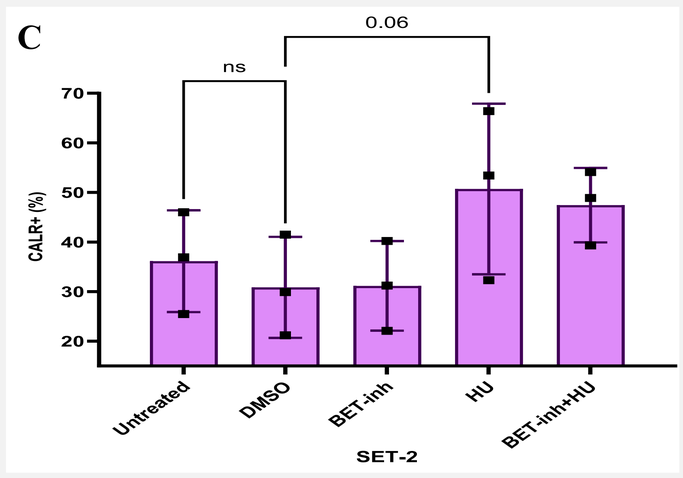


Figure A illustrates the percentage of CD47-positive SET-2 cells following 24 hours of treatment under different conditions. Figure B depicts the mean fluorescence intensity of CD47 on SET-2 cells. The blue and orange arrows indicate the negative population, which shows a marked increase after treatment with the BET inhibitor alone and in combination with hydroxyurea, respectively. * p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001. Data from n=3 experiments.

- Treatment with BET inhibitor alone significantly decreased CD47 expression in SET-2 cells after 24 hours of incubation, dropping from 96% to 76% (p<0.0001). No significant difference was seen between the untreated and DMSO controls (Figure A).

- When combined with hydroxyurea, the BET inhibitor showed similar reductions in CD47 expression from 96% to 78% (p < 0.0001) (Figure B).



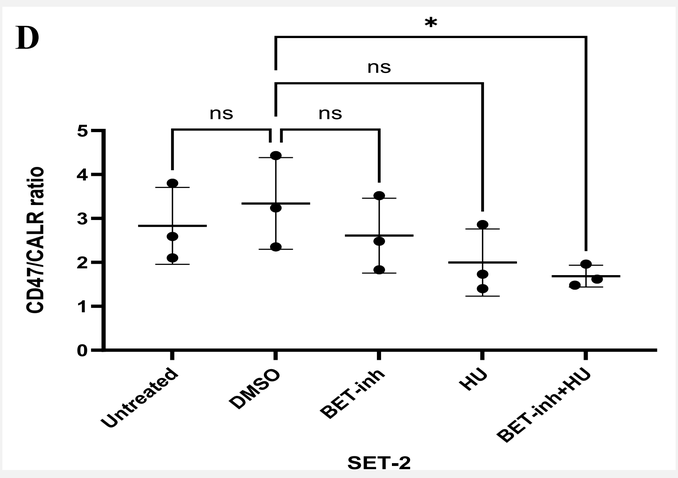


Figure C shows the percentage of CALR-positive SET-2 cells after 24 hours of treatment under various conditions. Figure D presents the CD47/CALR ratio in SET-2 cells following exposure to different agents.

- CALR expression increased with hydroxyurea treatment alone (51%) and with the combination of hydroxyurea and the BET inhibitor (41%) compared to the DMSO control; however, these changes were not statistically significant (Figure C).

- In contrast, the CD47/CALR ratio was significantly reduced with the combined treatment, decreasing from 3.3 in the DMSO control to 1.6 (p<0.05)(Figure D).

Conclusion

- These findings indicate that the BET inhibitor significantly reduced expression of the anti-phagocytic signal CD47 in the MPN cells, whereas hydroxyurea primarily influences the expression of pro-phagocytic signal CALR.
- The marked reduction in the CD47/CALR ratio with the combined treatment underscores the potential for novel combination strategies in MPN therapy.
- Future studies will primarily investigate the effects of BET inhibition on macrophage-mediated phagocytosis/leukaemia cell removal & its impact on macrophage activity levels in co-culture systems with MPN cells.