Investigating immunosurveillance by macrophage for potential therapeutic strategies in Myelodysplastic Syndrome (MDS)

Anya Squires[1], Niloofar Amirian[1], Claire L Walker[1], Ciro R Rinaldi[1], [2] & Matthew J Simmonds[1]

1 = School of Natural Sciences, College of Health & Science, University of Lincoln, Lincoln, UK & 2 = United Lincolnshire Teaching Hospitals, Lincoln, UK

Keywords: *Myelodysplastic Syndrome, Macrophage, Haematological Malignancies, Immunotherapies, CD47, Polarisation, Immune Evasion*

Current Challenges in Treating MDS

- Myelodysplastic syndromes (MDS) are a group of myeloid malignancies characterised by disturbances in maturation & differentiation of haematopoietic stem cells [1].
- Patients present with an array of features including petechiae and easy bruising (see Figure 1)
- Current therapies revolve round chemotherapy & demethylating agents, such as azacitidine, however many patients don't respond to these therapies, highlighting the desperate need for new treatment approaches.

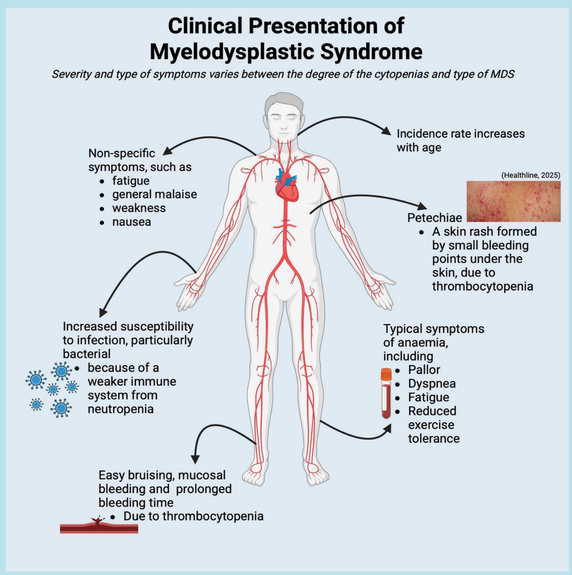
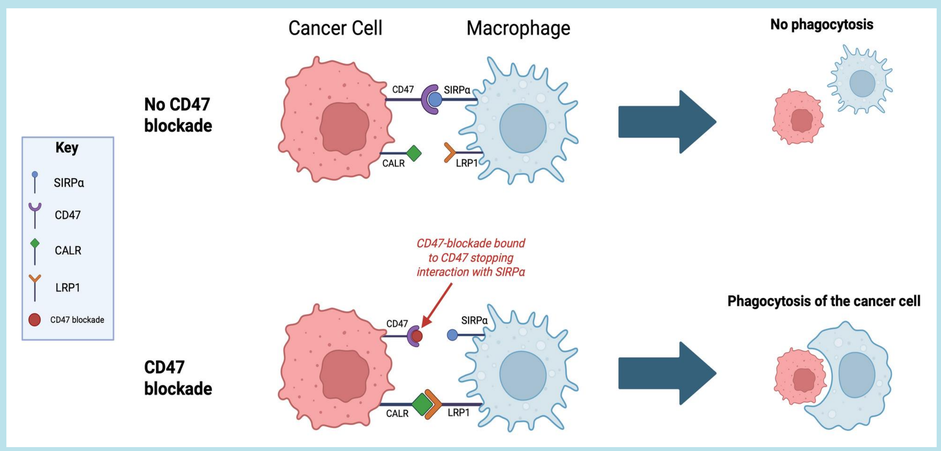


Figure 1. Clinical presentation of myelodysplastic syndrome

Targeting CD47 as a Novel Therapeutic Approach for MDS

- New therapies are being developed to block the CD47-SIRP-α inhibitory phagocytic axis which becomes more prominent as MDS disease progresses [2] and enhances the patients own macrophage's ability to phagocytose malignant cells



- Despite promising pre-clinical data, clinical trials for the CD47-blockade in MDS have shown less success, leaving questions over which stages of MDS disease to target these therapies in and whether changes in macrophage inflammatory status as MDS progresses could impact treatment success.

Aim

Investigate the impact of macrophage polarisation on CD47-blockade efficacy in MDS cell models using an indirect macrophage and cancer cell in vitro co-culture model

Methodology

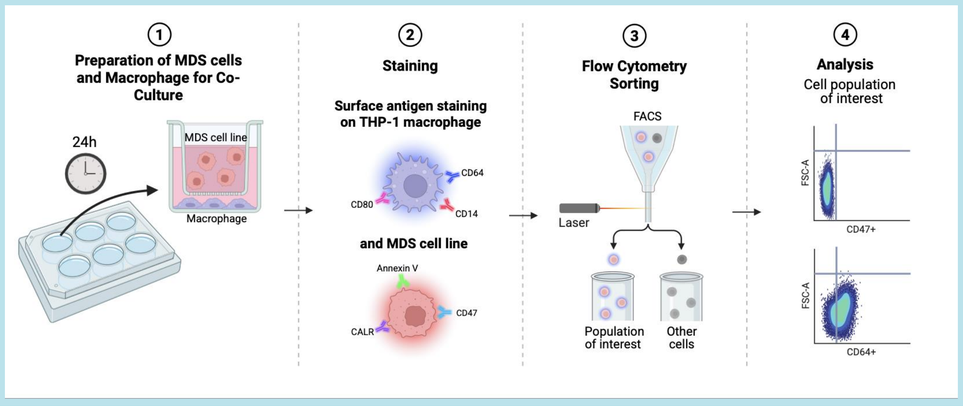
We initially determined how different combinations of standard therapies & anti-CD47 blockade impact upon cell line models of MDS (SKM-1) using flow cytometry.

↓

A method for generating monocyte derived M1 pro-inflammatory/anti-tumour & M2 anti-inflammatory/pro-tumour macrophage from THP-1 cells to mimic changes in the tumour microenvironment as MDS progresses.

↓

An indirect co-culture method was then applied to imitate the tumour microenvironment with & within the presence of novel immunotherapeutics.



Presence of pro-inflammatory (M1) THP-1 Macrophage in co-culture with SKM-1 cells treated with CD47 blockades preserves SKM CD47 expression

Treatment of SKM-1 cells with CD47 blockade without macrophage presence causes a significant reduction in surface expression of CD47 (A). In addition, a significant reduction in CD47 expression was noted in both CD47-blockade alone, and combination therapy with standard therapy (Azacitidine), compared to standard therapy alone (0.8%) ($p<0.01$) (Figure 2).

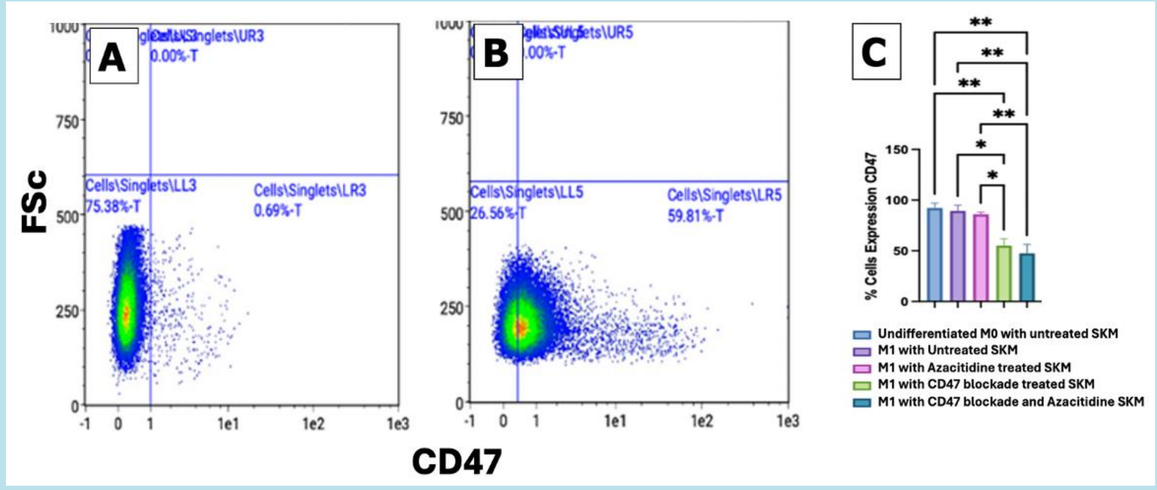


*Figure 2: Co-culture of SKM-1 cells treated with CD47-Blockade in conditions (A) without macrophage and (B) in a THP-1 M1 macrophage co-culture system. A: SKM-1 cells not subject to co-culture treated with CD47-blockade expressed minimal CD47 (<1% of live cells). B: SKM-1 cells subject to both CD47 blockade & co-culture with M1 THP-1 macrophage retained CD47 expression in ~60% of live cells. C: Summary of the significant differences found in SKM-1 cells CD47 expression in both CD47 vs mainstay therapy azacitidine alone. *=p<0.05, **=p<0.01.*

However, when SKM-1 cells were treated with CD47 blockade in the presence of pro-inflammatory M1 macrophage in a co-culture system, the led to the retention of CD47 expression in ~60% of cells (B) (Compared to <1% CD47 in SKM-1 cells alone when treated with CD47 inhibitor) (Figure 2), showing the presence of macrophage substantially altered CD47-blockade's function.

Co-culture with SKM-1 cells upregulates expression of both pro- & anti-inflammatory macrophage markers

To determine whether presence of MDS SKM-1 cells were also manipulating the macrophage to become more anti-inflammatory/ or pro-tumorogenic, M0 macrophages (who have not yet differentiated to either a pro- or anti-inflammatory state) were co-cultured with SKM-1 cells and markers of M1/M2 status were examined.

The M1 macrophage marker, CD80 increased from 24% on M0 macrophage (in absence of SKM-1 cells) to 64% when in co-culture with SKM-1. Equally expression of anti-inflammatory M2 macrophage specific antibodies CD163 and CD206 was markedly increased, with levels of these markers doubling during the co-culture experiments (Figure 3).

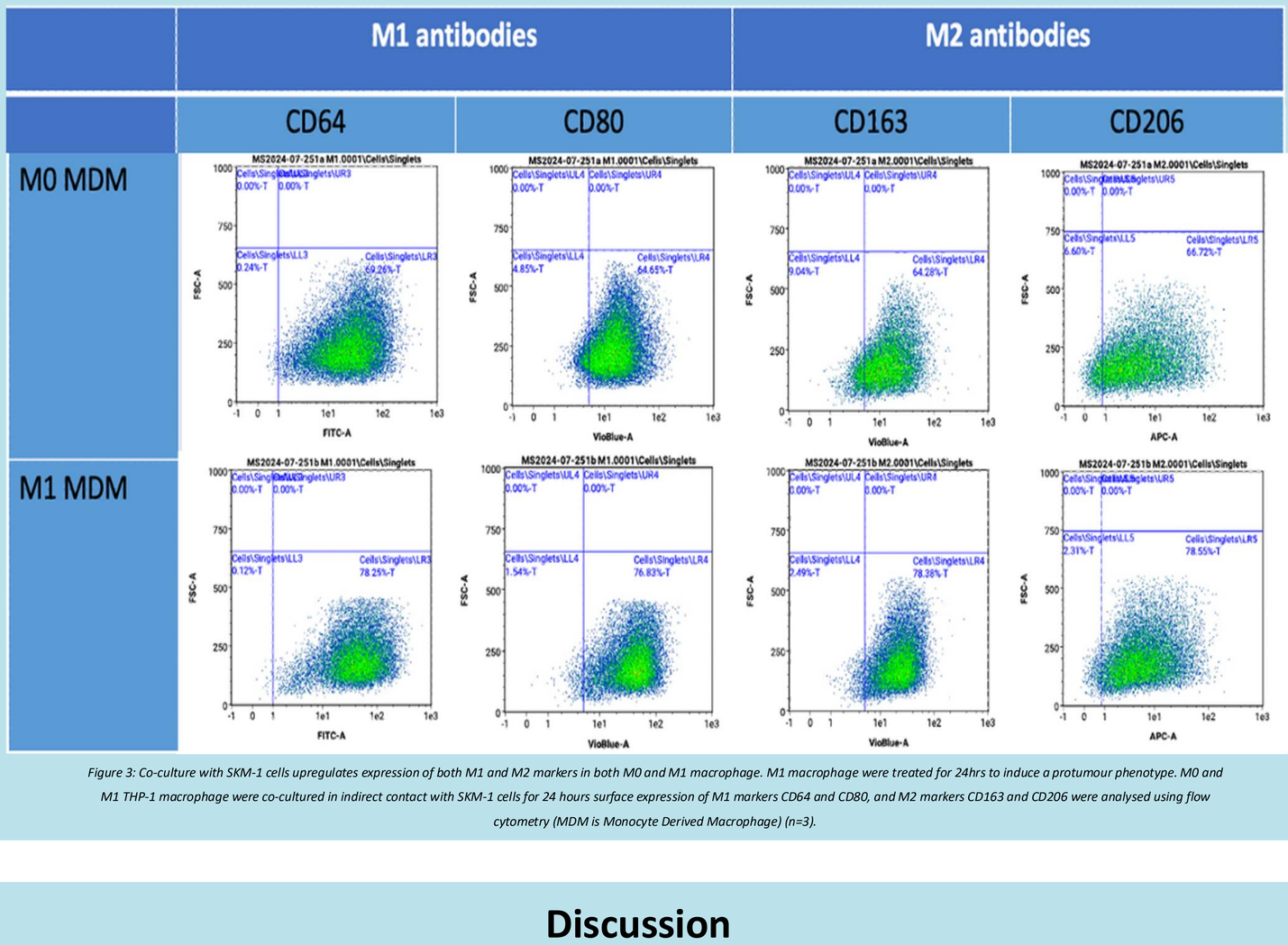


Figure 3: Co-culture with SKM-1 cells upregulates expression of both M1 and M2 markers in both M0 and M1 macrophage. M1 macrophage were treated for 24hrs to induce a protumour phenotype. M0 and M1 THP-1 macrophage were co-cultured in indirect contact with SKM-1 cells for 24 hours surface expression of M1 markers CD64 and CD80, and M2 markers CD163 and CD206 were analysed using flow cytometry (MDM is Monocyte Derived Macrophage) (n=3).

Discussion

- These findings underscore the influence of the tumour microenvironment on the efficacy of CD47 blockade therapeutic response in MDS. This suggests that macrophage inflammatory status plays a key role in treatment efficacy & potentially explains the failure of high risk MDS patients to respond to this novel therapeutic.
- Notably, tumour cells induce a mixed macrophage phenotype, characterised by the upregulation of both anti- and pro-oncogenic markers, suggesting that a patients macrophage status could impact treatment success.
- This could potentially provide insights into which patients would benefit from therapeutics targeting the SIRPα-CD47 axis when designing future trials & emphasises the necessity of considering the evolving tumour microenvironment when developing therapeutic strategies for MDS.

Acknowledgements: We would like to thank the Institute of Biomedical Science (IBMS) & University of Lincoln Pump Priming Scheme for funding this work

References:

1 Dotson, J.L. and Lebowicz, Y. (2022). *Myelodysplastic syndrome*. Available at: https://www.ncbi.nlm.nih.gov/books/NBK534126/
2 Boasman, K, Walker CL, Simmonds MJ & Rinaldi CR, Haematologia, 2025, *In Press*