# UNDERSTANDING IMMUNE CELL DYSFUNCTION IN THE MYELOPROLIFERATIVE NEOPLASMS (MPNS)

**Authors**
Aya Hammad supervised by Dr Alyssa Cull & Dr David Kent

**Acknowledgements**
This project was completed thanks to the Laidlaw Undergraduate Research & Leadership Programme at the University of York. Thank you to my supervisors: Dr Alyssa Cull and Dr David Kent who believed in me and supported me endlessly. Finally, I am eternally grateful to Lord Laidlaw for his generosity without which I would not be where I am today.

## BACKGROUND

- Myeloproliferative neoplasms (MPNs) are a group of rare bone marrow disorders characterised by the rapid and abnormal growth of blood cells (Cancer Research UK, 2020).
- Mutations in Ten-Eleven-Translocation 2 (TET2) are frequently observed in patients with MPNs (Ko et al., 2011).
- TET2 is a gene that acts as a tumour suppressor and regulates blood cell production, predisposing some individuals to developing a myeloid cancer (Ko et al., 2011).
- Patients with more advanced forms of MPNs are at a higher risk of developing life-threatening leukaemia (Brabrand and Frederiksen, 2020).
- TET2 is frequently mutated in MPN patients as seen in Figure 1.

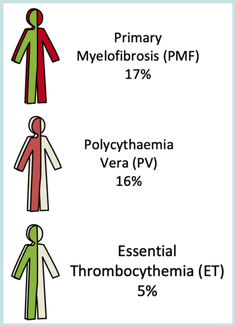



*Figure 1. Compare the percentage of patients with TET2 mutations between the 3 MPNs.*

## INTRODUCTION

- High inflammation levels in MPN patients may contribute to disease development (Øbro et al., 2020).
- A combination of mutations in genes including TET2 and JAK2 contribute to MPNs.
- Contributions of macrophages to this inflammatory environment remain unclear. The mechanism for Stem Cells with these mutations is known and shown in Figure 2.
- TET2 is the most highly expressed TET enzyme in mouse macrophage differentiation (Tefferi et al., 2009).
- Tet2 knockout mouse models are used to study TET2 mutations and compare them with the normal genotype: Wild-Type (WT)

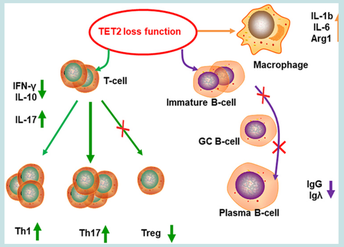



*Figure 2 – TET2 functions in immune regulation. TET2 can regulate T cell differentiation. Because of TET2 loss of function, cytokines secretion changed. TET2 can inhibit inflammation in macrophages, TET2 mutated macrophages produce more inflammatory factors, such as IL-1b, IL-6, and arginase1.*

*Image taken from Feng et al., 2019*

## OBJECTIVES

We have conducted this primary research study to further our understanding of the mutant immune cell populations using a Tet2 knockout mouse model.

*The specific aims of this report are as follows:*

- Establish a basic protocol used to identify various immune cell populations in different mouse tissue samples, and devise gating strategies to identify the macrophage populations in each tissue type
- Compare the frequencies of macrophages present in the liver, spleen, and bone marrow in Tet2 knockout and WT mice.
- Look at macrophage cell surface marker expression to compare WT and Tet2 knockout macrophages in mice.

## METHODOLOGY

**STEP 01 Prepare cell suspensions from tissue**

- Mouse tissue samples were collected from the spleen, liver and bone marrow of two mouse models: WT and Tet2 Knockout as summarized by Figure 3.
- The tissue samples were processed using a standard lab protocol.

**STEP 02 Prepare for flow cytometry**

- The prepared cell suspensions for all tissue samples were treated with antibodies
- The antibodies used for each tissue sample were selected according to the specific properties (cell surface markers) of the targeted immune cell populations.

**STEP 03 Assess cell surface marker status using flow cytometry**

- Data was acquired on a CytoFLEX Flow Cytometer.
- The compensation and data analyses were performed using FlowJo Software.

**STEP 04 Analyse flow cytometry data**

- The macrophage cell populations for the different tissue samples in each of the panels were identified using a sequential gating strategy as shown in Figure 4.
- The gating strategies relied on known cell markers specific to macrophages.
- We used the Mean Fluorescence Intensity (MFI) as a rough indicator of protein abundance on the surface of cells.

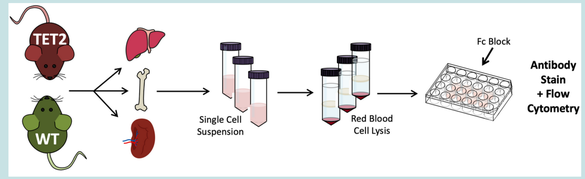
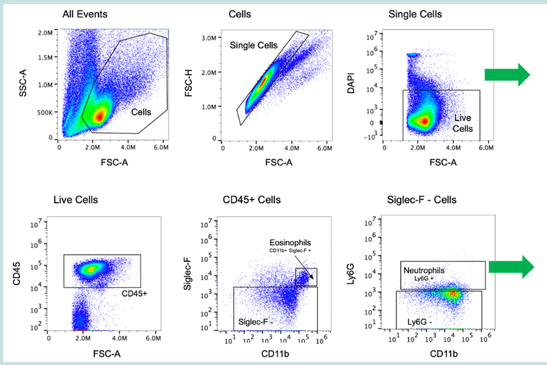



*Figure 3 – Summary of lab protocol used to prepare cell suspension for flow cytometry analysis. Mice with the absence of the TET2 gene (Tet2 Knockout model) were used and Wild-Type WT mice were the control.*



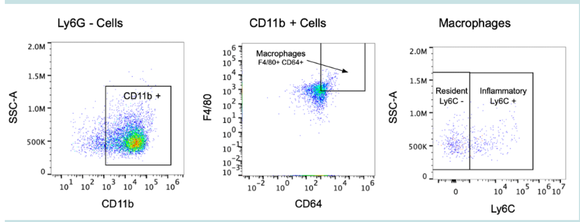




*Figure 4 - An example of the gating strategy used for the liver samples. This was used to identify and isolate the immune cells in question; liver macrophages in this case.*

## ANALYSIS

- In a Tet2 knockout mouse model, the liver would be expected to have higher levels of inflammation.
- The liver resident macrophages may elicit a different response to increased inflammation compared to other tissues (Shan & Ju, 2020).
- The healthy liver contains macrophages known as Kupffer cells which have crucial functions in maintaining homeostasis (Krenkel & Tacke, 2017).
- In an injured liver, monocyte-derived macrophages (MDMs) rapidly accumulate as they are inflammatory-mediated cells (Krenkel & Tacke, 2017).
- The increased inflammation supports the higher frequency of macrophages observed in the liver samples as the MDMs would infiltrate the liver.
- Both macrophage populations can expand in the liver (Krenkel & Tacke,

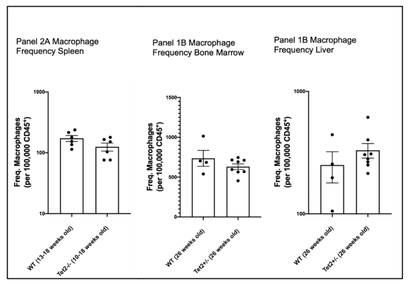



*Figure 5 - The frequency of macrophages in the liver, spleen and bone marrow amples in WT and Tet2 knockout (Tet2 +/-) models..*

- Figure 5 shows the frequency of macrophages in each of the tissue samples
- In the bone marrow and the spleen, there is a lower macrophage frequency in the Tet2 knockout samples than in the WT mice samples.
- This is not the case in the liver.
- The bone marrow panel had the highest frequency of macrophages for both Tet2 knockout and WT.

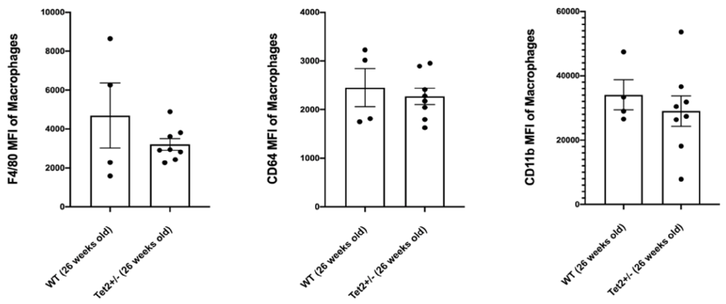



*Figure 6 shows The MFI values for the Liver Samples for the cell markers called: CD11b, F4/80 and CD64.*

- Figure 6 shows that Tet2 mice displayed a lower MFI for all the cell markers in the liver samples
- F4/80 is highly expressed in Kupffer cells compared to MDMs (Lin et al.,2005)
- High levels of infiltration of the F4/80-low MDMs might explain why there is lower F4/80 expression in Tet2 knockout samples in the liver compared to WT.
- MDMs have higher expression of CD11b compared to Kupffer cells (Shan & Ju, 2020).
- We would expect the CD11b MFI for Tet2 knockout in the liver to be higher but this was not the case according to Figure 6.
- Further investigation would be needed to clarify the role of CD11b expression in the Tet2 knockout microenvironment.

**Significance of cell surface markers**

- CD11b plays an important role in myeloid cell migration within sites of inflammation (Panni et al., 2019).
- Thus, CD11b expression should be higher in Tet2 knockout mouse models due to increased inflammation.
- This is the case according to our results for the spleen and bone marrow where the CD11b MFI was higher in Tet2 samples
- CD11b expression was lower in the liver samples in Tet2 knockout mice than in WT mice.

## SUMMARY

- We identified cells with cell surface marker expression features consistent with macrophages.
- Our study confirmed the variability in the frequencies of macrophage populations in Tet2 knockout and WT mouse models.
- The higher frequency of macrophages observed in the Tet2 knockout liver samples is consistent with the expected inflammation.
- This is a good model for the loss of Tet2 as the human TET2 gene is frequently observed to be mutated in patients with diverse myeloid malignancies (Ko et al., 2011).
- We furthered our understanding of the variations in macrophage frequency in the absence of the TET2 gene.
- We provided a better understanding of the expression of protein cell-surface markers in Tet2 knockout and WT mouse models.

## CONCLUSION

- Understanding the different levels of expression of cell surface markers can help us understand and compare the inflammatory changes taking place in mutated macrophages.
- This may help future studies in associating specific macrophage changes with their behaviour in the diseased state (MPNs).
- We can begin to examine the role of resident and recruited macrophages and other immune cells in murine models of MPNs using the methodology of this study.
- Understanding how cells behave and interact with their microenvironment differently can help us develop new targeted therapies for MPNs.

## References

Brabrand, M. and Frederiksen, H., (2020). Risks of Solid and Lymphoid Malignancies in Patients with Myeloproliferative Neoplasms: Clinical Implications. Cancers, 12(10), p.3061.
Cancer Research UK (n.d.). Myeloproliferative neoplasms | Other conditions | Cancer Research UK. [online] www.cancerresearchuk.org. Available at: https://www.cancerresearchuk.org/about-cancer/other-conditions/myeloproliferative-neoplasms. [Accessed 15 January 2022].
Feng, Y., Li, X., Cassady, K., Zou, Z. and Zhang, X., 2019. TET2 Function in Hematopoietic Malignancies, Immune Regulation, and DNA Repair. Frontiers in Oncology, 9.
Ko, M., Bandukwala, H. S., An, J., Lamperti, E. D., Thompson, E. C., Hastie, R., Tsangaratou, A., Rajewsky, K., Koralov, S. B., & Rao, A. (2011). Ten-Eleven-Translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. Proceedings of the National Academy of Sciences of the United States of America, 108(35), 14566–14571. https://doi.org/10.1073/pnas.1112317108
Krenkel, O., & Tacke, F. (2017). Liver macrophages in tissue homeostasis and disease. Nature Reviews Immunology, 17(5), 306–321. https://doi.org/10.1038/nri.2017.11
Øbro NF, Grinfeld J, Belmonte M, Irvine M, Shepherd MS, Rao TN, Karow A, Riedel LM, Harris OB, Baxter EJ, Nangalia J, Godfrey A, Harrison CN, Li J, Skoda RC, Campbell PJ, Green AR, Kent DG. Longitudinal Cytokine Profiling Identifies GRO-α and EGF as Potential Biomarkers of Disease Progression in Essential Thrombocythemia. Hemasphere. 2020 May 21;4(3):e371. doi: 10.1097/HS9.0000000000000371. PMID: 32647796; PMCID: PMC7306314.
Shan, Z., & Ju, C. (2020). Hepatic Macrophages in Liver Injury. Frontiers In Immunology, 11. https://doi.org/10.3389/fimmu.2020.00322
Tefferi, A., Pardanani, A., Lim, K-H., Abdel-Wahab, O., Lasho, T.L., Patel, J., Gangat, N., Finke, C.M., Schwager, S., Mullally, A., Li, C-Y., Hanson, C.A., Mesa, R., Bernard, O., Delhommeau, F., Vainchenker, W., Gilliland, D.G. and Levine, R.L. (2009). TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. Leukemia, 23(5), pp.905–911.