P799 SPATIAL ANALYSIS IDENTIFIES A SPECTRUM OF IMMUNE DYSREGULATION IN ACQUIRED BONE MARROW FAILURE CONDITIONS

**Topic:** 11. Bone marrow failure syndromes incl. PNH - Biology & Translational Research

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**Background:**

Poor Graft Function (PGF), manifested by multilineage cytopenias with complete donor chimerism post allogeneic stem cell transplantation, and acquired Aplastic Anaemia (AA) are acquired bone marrow failure syndromes. Both are thought to result from T cell activation resulting in excessive interferon-γ (IFNγ) production, which in turn suppresses haematopoiesis. In AA, this can promote clonal stem cell selection and potentially progression to myeloid malignancy. Despite similarities in the clinical presentation and proposed mechanism, no studies to date have compared the immunobiology of the bone marrow (BM) microenvironment in these conditions.

**Aims:**

To examine the immune microenvironment of the BM in archival AA and PGF BM trephines and identify common immunopathologies.

**Methods:**

Archival BM trephines were sourced from patients with PGF (n=20), AA at Diagnosis (AA_DX) (n=15), AA at Progression (AA_PROG) to MDS/AML (n=15) and normal controls (NC) (n=20). 6 x 300µm regions identified by dual CD3/CD45 immunofluorescent staining were analysed per BM trephine using NanoString GeoMX™ digital spatial profiling for the expression of 57 proteins with an immunology focused panel. Data was analysed using a Limma-Voom bioinformatics pipeline.

**Results:**

Significant dysregulation was identified across multiple proteins. Overall, 22 proteins had significantly different expression in AA_DX samples (13 up, 9 down) compared to NC with AA_PROG and PGF having 8 (4 up, 4 down) and 14 (7 up, 7 down) respectively. As would be expected given the hypocellular BM, expression of CD45 was significantly reduced compared to NC across AA_DX (adj P = 5.413e-10), AA_PROG (adj P = 8.299e-8) and PGF (adj P = 8.634e-9) groups.

When AA_DX patients were stratified into those who progressed (n=6) vs those who did not (n=9), or when AA_DX vs AA_PROG was compared in patients with matched samples (n=6) there were no significantly differentially expressed proteins identified. This suggests that while AA provides an immune microenvironment that is permissive to MDS/AML pathogenesis, it may not directly impact on clonal evolution.

Total monocytes were unchanged by CD11c expression. However, AA and PGF samples exhibited upregulation of CD163 compared to NC (AA_DX adj P = 4.5e-6; AA_PROG adj P = 1.2e-5; PGF adj P = 1.58e-8) suggesting an increase in monocyte/macrophage lineage cells. Expression of CD66b was also downregulated in AA_DX (adj P = 8.459e-24), AA_PROG (adj P = 1.193e-10) and PGF (adj P = 2.244e-14) suggesting a reduction in non-classical monocytes, neutrophils or granulocytic MDSCs.

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Importantly, VISTA was downregulated across AA_DX (adj P = 6.84e-27), AA_PROG (adj P = 1.1e-5) and PGF (adj P = 1.58e-8) and STING was downregulated in AA_DX compared to NC (adj P = 6.139e-13) with a trend towards reduced expression in PGF. Decreased expression of these key immunoregulatory proteins may have wide ranging effects on BM resident macrophages, dendritic cells, MDSCs and T cells leading to reduced immunoregulation and increased T cell activation/IFNγ production, ultimately resulting in stem cell depletion. Data on cell specific expression is currently being obtained to refine this model.

**Summary/Conclusion:**

Spatial analysis demonstrated that patients with AA and PGF exhibit similar patterns of protein expression likely resulting in a BM immune microenvironment of decreased immunoregulation. AA_DX samples exhibited a greater degree of dysregulation of multiple markers compared to PGF suggesting that these diseases represent a spectrum of immune dysregulation.