# Abstract: P1486

# Title: HYDROXYUREA MODULATES VLA-4 BINDING TO VCAM-1 THROUGH ERYTHROID SIGNALING PATHWAYS INDEPENDENT OF ADHESION RECEPTOR EXPRESSION IN PRIMARY CULTURES OF SICKLE ERYTHROBLASTS

**Abstract Type: Poster Presentation**

**Topic: Sickle cell disease**

**Background:**

Sickle cell disease (SCD) is a rare, red blood cell (RBC) disorder producing sickle hemoglobin (HbS) that polymerizes during deoxygenation. Subsequent RBCs form rigid, sickle-shaped cells that directly promote vaso-occlusive episodes (VOEs). Repetitive oxygenation-deoxygenation cycles destabilize RBC membranes making them prone to hemolysis which triggers the production and premature release of RBCs in circulation to meet the oxygen demands of the body. Integrins are highly expressed on erythroid precursors during normal hematopoiesis to anchor blood cells in the bone marrow and, conversion to a low affinity conformational state by erythroid signaling pathways has been suggested as a plausible mechanism to prematurely release RBCs during anemic stress in SCD, independent of adhesion receptor expression. Reticulocytes (immature RBCs) are major contributors to VOEs in SCD by abnormally interacting with circulating blood cells and the vascular endothelium.

Hydroxyurea (HU) decreases the adhesive potential of circulating blood cells by reducing reticulocyte counts, decreasing adhesion receptor expression and, modulating key erythroid signaling pathways to reduce HU-induced adhesive interactions. Very late antigen-4 (VLA-4), the best characterized adhesion receptor in SCD, is increased on sickle reticulocytes during VOEs and decreased during HU therapy. Like other integrins, VLA-4 is functionally regulated by cell signaling pathways to modulate activity to bind a wide variety of ligands elevated in the SCD micro-environment. Nonetheless, erythroid signaling pathways involved in regulating VLA-4 activity in SCD is poorly understood.

**Aims:**

The aim of this study was to determine whether HU modulates erythroid signaling pathways in sickle erythroblasts to reduce VLA-4 binding to VCAM-1 independent of VLA-4 expression.

**Methods:**

Hematopoietic stem cells (HSCs) were isolated from SCD patients and matured to erythroblasts (EBs) in appropriate cell culture media followed by treatment with 50mcM HU. Primary EB cultured cells were perfused through microfluidic channels coated with vascular cell adhesion molecule-1 (VCAM-1). An adhesion index (cells/mm2) was established for each sample by quantifying adherent cells within a standard viewing area. Flow cytometric analyses assessed VLA-4 expression (CD49d+) and activation (CD29+) on EBs (CD235a+CD71+), and cell viability (7-AAD). The non-parametric Wilcoxon matched pairs signed rank test was used to test the statistical differences between groups. A p-value $< 0.05$ was considered statistically significant.

**Results:**

Untreated EB adhesion to VCAM-1 (295 to 878 cells/mm2) and the effect of HU treatment on cultured EBs (0.20- to 0.65- fold change) varied from patient-to-patient (n=9). HU significantly decreased VLA-4 binding to VCAM-1 (n=9; p=0.004). Flow cytometric analysis (n=4) confirmed EB culture viability and purity. HU treatment did not alter VLA-4 expression ($p>0.9999$) or activation (p=0.4375).

**Summary/Conclusion:**

Prior studies established whole blood (WB) adhesion to VCAM-1 at steady state identifies SCD patients with severe disease phenotypes and, strongly correlates with reticulocyte counts. VLA-4 is the only known adhesion

receptor on RBCs that bind endothelial VCAM-1. In this study, we observed a highly variable range of EB adhesion to VCAM-1 that may represent clinically relevant phenotypic differences observed in this study. Our data also reveals that VLA-4 expression is unchanged in HU-treated EBs although VLA-4 binding to VCAM-1 was significantly decreased indicating HU modulates adhesion independent of adhesion receptor expression. Interestingly, VLA-4's active confirmation state was not significantly altered by HU despite effects on functional binding in our flow adhesion assay. VLA-4 exists in multiple activation states, and it is likely that CD29 may not be appropriate to assess VLA-4 activity that corresponds to VLA-4 binding to VCAM-1. Ongoing studies aim to further investigate these findings and, reveal erythroid signaling pathways that modulate VLA-4 activity in sickle RBCs to develop novel therapies for patients living with SCD.

**Keywords:** Vasoocclusive crisis, Sickle cell adhesion, Integrin activation, VCAM-1