# Exploring Experimental Hematology: April 2020 (Volume 84)

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## Exploring Experimental Hematology: Tandem P-selectin glycoprotein ligand immunoglobulin (TSGL-Ig) prevents lung vaso-occlusion in sickle cell disease mice

In this issue of Simply Blood, Derek Chan is exploring Experimental Hematology by highlighting and deconstructing one of the journal's latest manuscripts on work led by first author Ravi Vats from the laboratory team of Dr. Prithu Sundd (Vats *et al*., 2020).

### How do vaso-occlusive episodes occur in sickle cell disease?

The clinical course for patients with sickle cell disease (SCD) often involves recurrent and unpredictable episodes of painful vaso-occlusive episodes (VOEs) that may precipitate life-threatening sequelae. While the molecular basis for SCD is well understood, the complex mechanisms underlying VOEs are not as well elucidated.

In these last two decades, several research groups have worked to characterize the contributions of both cellular and soluble components to the cascade of events leading to VOEs. Notably, work using intravital microscopy has shown that the predominant in vivo interactions in the setting of a VOE occur between circulating sickled red blood cells, adherent leukocytes

and platelets, with these being importantly dependent on the expression of P- and E-selectin receptors (Turhan *et al*., 2002; Bennewitz *et al*., 2017).

**Figure 1:** (Left) Ravi Vats and (Right) Dr. Prithu Sundd.

**How can VOEs be therapeutically targeted?**

While a glycomimetic "pan-selectin" small molecule inhibitor (subsequently found to be E-selectin dominant) failed important endpoints in a recent Phase 3 trial, a P-selectin monoclonal antibody encouragingly lowered rates of VOEs and associated adverse events (Ataga *et al*., 2017). Several lines of experimental evidence have also accrued in support of a rationale to target P-selectin interactions, including observations that P-selectin dependent neutrophil-platelet-erythrocyte aggregates are significantly elevated in SCD patient blood (Dominical et al., 2014), that related microembolisms are resolved by anti-P-selectin treatment in vivo (Bennewitz *et al*., 2017) and that P-selectin deficient mice are protected against VOEs (Bennewitz *et al*., 2020).

**Reasons to read this paper:**

Vats *et al*. (2020) now provide additional intravital evidence that a tandem anti-P-selectin ligand immunoglobulin (TSGL-Ig) can also prevent lung VOEs in a SCD mouse model. Particularly compelling are the videos captured showing the markedly different levels of vascular patency in lungs between mice receiving a control antibody versus those receiving an anti-P-selectin ligand immunoglobulin that acts as a decoy receptor in the setting of a VOE precipitating event.

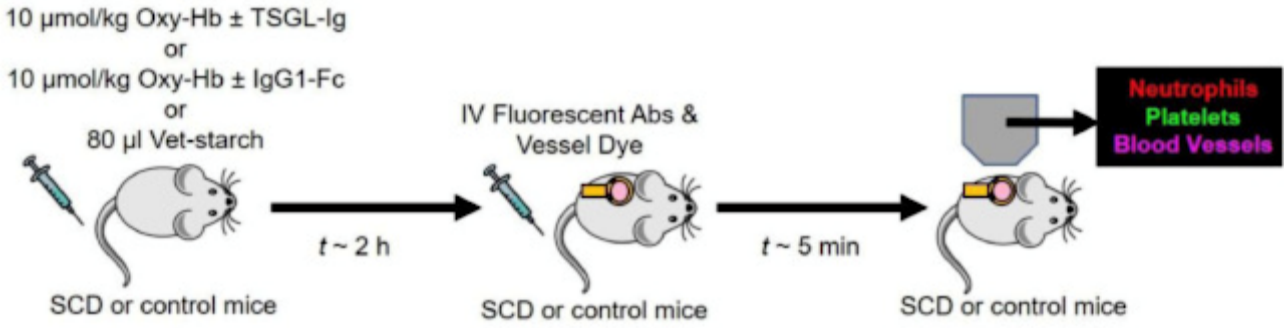



**Figure 2:**; Experimental schematic of modeling and monitoring vaso-occlusive events in the lungs of a SCD mouse model (Adapted from Vats et al., 2020).

**Strategy used in paper:**

Vats *et al.* (2020) modeled the precipitation of lung VOEs in humanized Townes SCD mice by way of intravenous administration of oxy-hemoglobin in the presence of either a TSGL-Ig

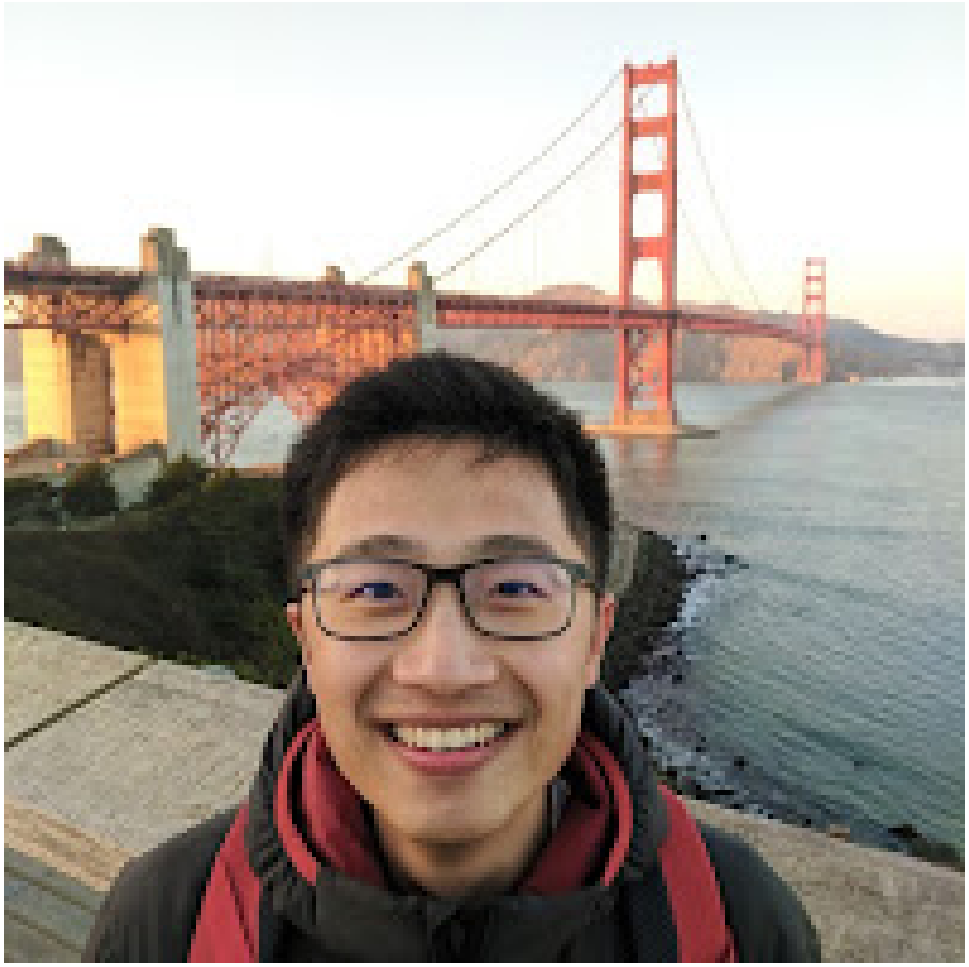
or an immunoglobulin control (IgG1-Fc). Mice were anesthetized 1 hour later for surgery and by 2 to 2.5 hours, they infused fluorescent dyes via the carotid artery to label the blood vessels, neutrophils and platelets for quantitative intravital lung microscopy for a maximum period of 30 minutes, capturing images from different fields of view and from a few mice per test group. Images were then processed, quantified for vaso-occlusions and compared on the basis of numbers identified per field of view.

**Figure 3:** Neutrophil-platelet aggregate (white circle) occluding lung arteriole bottle-neck in a SCD mouse model in the presence of IgG1-Fc. (Right) Absence of neutrophil-platelet aggregates in lung arteriole in a SCD mouse model in the presence of TSGL-Ig. Labels are as follows: Neutrophils (red), platelets (green), microcirculation (purple). (Movies adapted from Vats *et al*., 2020)

**Future considerations:**

Altogether, this work supports the approach of disrupting P-selectin interactions as a way to prevent VOE cascades, results which are consistent with those obtained by the SUSTAIN trial. In the future, it may be important to conduct a direct comparison between these two types of antibodies and to see if there are any indirect disadvantages to disrupting P-selectin mediated neutrophil adhesions, such as in cases of concurrent bloodstream infections in affected patients. Overall, this is an important piece of work that sheds light on a way to potentially treat and manage VOEs in patients with SCD.

*Written by:*



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