



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

The Variable Heavy Chain Antibody Syn-VWFA1 Inhibits the Interaction of Platelets to Von Willebrand Factor in Solution but Not to Immobilized Von Willebrand Factor Thereby Inhibiting 3-Dimensional but Not 2-Dimensional Thrombus Formation

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Neutralizing the interaction between GP1b α and von Willebrand Factor (VWF) is an attractive strategy for treatment and prevention of platelet-VWF agglutinate formation in von Willebrand disease type 2B and thrombotic thrombocytopenic purpura. The aim of this study was to characterize a llama-derived variable heavy chain antibody (VHH) targeting the A1 domain of active VWF (Syn-VWFA1), in terms of its interference with platelet VWF interaction.

Syn-VWFA1 (1.5 μ M) abolished platelet binding to ristocetin-activated VWF (native and Haemate® P VWF) and R1306W VWF (VWF type 2B) in a flow-cytometric platelet function assay (Figure 1). Moreover, Syn-VWFA1 dose-dependently inhibited ristocetin-activated VWF and R1306W VWF platelet agglutination, but not collagen- and ADP-induced platelet aggregation. Under flow conditions, Syn-VWFA1 (1.8 μ M) did not affect adhesion of platelets to collagen at high shear. In the shear- and collagen-dependent PFA-200, Syn-VWFA1 prolonged the closure time, but only at high concentrations (>3 μ M), while GPIIb binding to immobilized VWF remained unaffected by Syn-VWFA1. Syn-VWFA1 increased the cleavage of VWF multimers by ADAMTS13. Furthermore, by applying deuterium exchange, we found a small region in the β 1 to α 1 loop (1288-1293) in the A1 domain of VWF, which is likely to constitute the nanobody binding site (Figure 2) and binding of Syn-VWFA1 to the A1 domain results in a conformational change exposing regions in the α 2 and α 3 helices.

In conclusion, Syn-VWFA1 dose-dependently blocks platelet-binding to activated VWF in solution, but not to (collagen-) immobilized active VWF. This implies that immobilization of VWF to collagen affects VWF conformation in such a way (1) that access of Syn-VWFA1 to its epitope in the A1 domain is hindered or (2) that conformational changes following Syn-VWFA1 binding are blocked, while allowing platelet binding. This implies that Syn-VWFA1 has potential treatment opportunities for patients with increased circulating active VWF, to prevent 3-dimensional thrombus formation (pathological platelet-VWF aggregate formation) but not 2-dimensional thrombus formation (leaving physiological hemostasis at sites of vascular injury intact).

Disclosures Huskens: Synapse Research Institute: Current Employment, Other: Synapse Research Institute is part of the Diagnostica Stago group. **Konings:** Synapse Research Institute: Current Employment. **Arce:** Loxo Oncology at Lilly: Current Employment, Current equity holder in publicly-traded company. **Roest:** Synapse Research Institute: Current Employment, Other: Synapse Research Institute is part of the Diagnostica Stago group. **De Groot:** Synapse Research Institute: Consultancy, Honoraria. **De Laat:** Diagnostica Stago: Other: Synapse Research Institute is part of the Diagnostica Stago group..

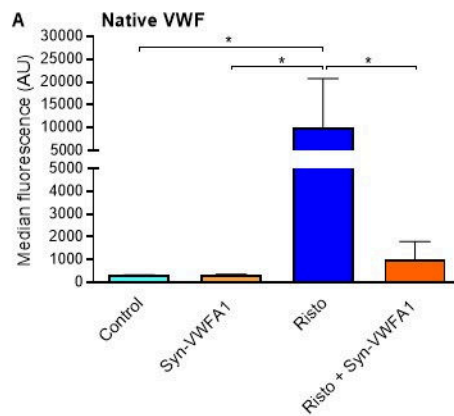


Figure 1. Syn-VWFA1 inhibits binding of VWF to platelets in solution. The inhibitory effect of the Syn-VWFA1 on the binding of native VWF, to platelets in whole blood, in the absence or presence of ristocetin, was determined by flow cytometry. WB was pre-incubated with buffer (control), Syn-VWFA1, ristocetin or the combination thereof. Data represent mean \pm SD of duplicate measurements of blood from 3 healthy donors. *** $P < 0.001$, * $P < 0.05$.



Figure 2. The crystal structure of the complex of the wild-type VWF A1 domain (green) and GPIIb/IIIa (orange) at 2.6 Angstrom Resolution (<https://doi.org/10.2210/pdb1SQ0/pdb>). The binding place of Syn-VWFA1 is in yellow, the AA that bind to GPIIb/IIIa in red.

Figure 1

<https://doi.org/10.1182/blood-2023-177887>