Results: RLYB211 markedly accelerated the elimination of HPA-1ab platelets in all participants versus placebo (half life of transfused platelets 0.32 hrs. vs. 65.29 hrs. respectively; p value <0.001; Fig. 1). Few treatment-emergent adverse events (TEAEs) were reported; 2 (headache and nausea) were possibly related to treatment, and both were in the RLYB211-treated participants. No participants had developed HPA-1a antibodies at 24 weeks (n=8).
Conclusion: These results establish proof of concept for prophylactic administration of RLYB211 to produce rapid elimination of HPA-1a-mismatched platelets and support its potential use for the prevention of HPA-1a alloimmunization and occurrence of FNAIT.

Disclosure Statements: MKj and JKK are stockholders of Prophylix AS, a Norwegian biotech company, which produced the study drug. MKj and JKK are currently consultants for Rallybio, which is the Sponsor of the study. ZB is employee of Rallybio. The remaining authors declare no competing financial interests.

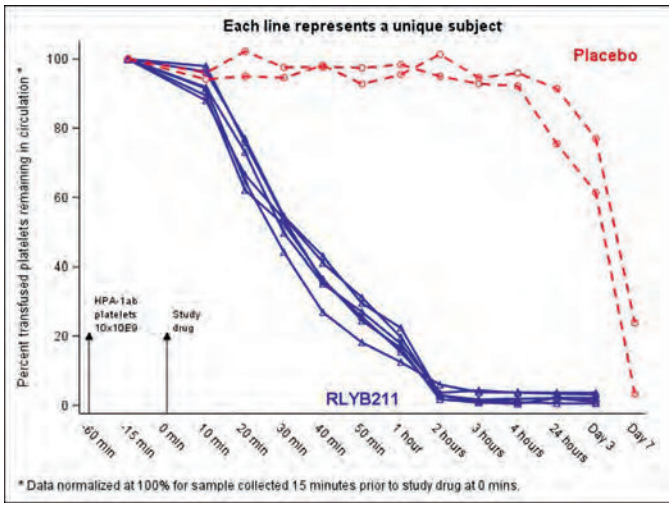

Fig. 1

VS-9-4

Flowcytometry-based phagocytosis assay of DAT+ erythrocytes as in-vitro surrogate for immune-mediated hemolysis

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Background: Extravascular immune hemolytic anemia (IHA) triggered by antibodies against red blood cell antigens is caused by tissue macrophages. Monocyte Monolayer Assay (MMA) has been used to assess IHA in vitro, but due to inherent variability and microscopic readout its diagnostic power is limited. Here we propose a flowcytometry-based approach to quantify Fc receptor-mediated phagocytosis by activated human blood monocytes incubated with antibody-coated erythrocytes (ACE).
Methods: CD14+ monocytes from human whole blood were primed with phorbol-12 myristate-13-acetate in order to trigger differentiation into macrophages (MA). Subsequent incubation with ACE, selected by presence of a positive direct antiglobulin test (DAT+), was carried out in 1:6 ratio (MA:ACE) for 1 h at 37°C. We quantified MA-ACE agglutinates by FACS upon staining with fluorophore-conjugated antibodies against CD14 and CD235a (Glycophorin A). Antibody-mediated agglutination was inhibited by co-incubation with IgG (Privigen). We used cytospin preparations to microscopically assess phagocytosis of MA-ACE agglutinates. In vitro hemolysis of ACE was determined by free hemoglobin.
Results: More than 600 experiments were performed with monoclonal and polyclonal antibodies of various blood group epitope specificites (Anti-D, -M, -N, -s, -Jka, -Ge and autoantibodies) to trigger in vitro phagocytosis (Fig. 1). The number of MA-ACE agglutinates proved to depend on many factors. In vitro hemolysis as measured by free hemoglobin is correlated with quantitative occurrence of MA-ACE phagocytosis. Microscopically assessed phagocytosis was also in good and robust concordance with FACS-based readout of MA-ACE agglutinates. Control of test parameters combined with FACS based assessment of MA-ACE phagocytosis provided reproducible and statistically robust surrogate of in vitro hemolysis.
Conclusion: Flowcytometry-based assessment (Fig. 2) of in vitro interaction between macrophages and antibody-coated erythrocytes allows qualification of cellular immune hemolysis. The power of our approach to also predict in vivo hemolytic potency of anti-red blood cell antibodies and circumvent limitations of MMA is currently being determined.

Disclosure Statements: None

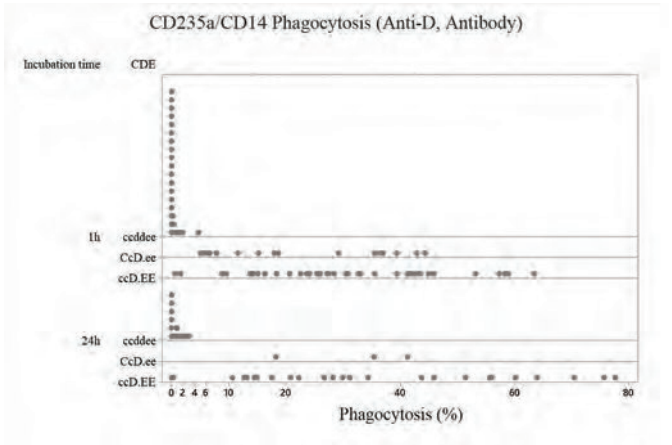

Fig. 1

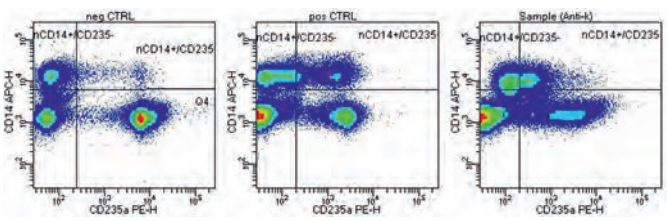

Fig. 2