Prophylactic Administration of HPA-1a–Specific Antibody RLYB212 Safely Prevents Fetal/Neonatal Alloimmune Thrombocytopenia Due to HPA-1a Incompatibility in Pregnant Mice

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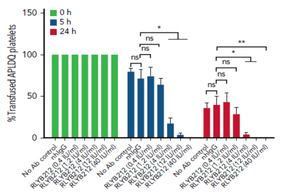
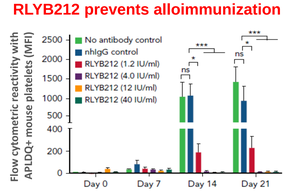
INTRODUCTION

- **Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) is a life-threatening bleeding disorder caused by maternal alloantibodies against paternally inherited antigens on fetal platelets. These antibodies, developed during pregnancy, cause fetal and neonatal thrombocytopenia, leading to intracranial hemorrhage in severe cases (10-20% of cases), with a mortality rate of 3-10%.**
- **25% to 50% of FNAIT cases occur without warning during gestation of the first pregnancy. FNAIT is often diagnosed after birth due to symptoms in the neonate.**
- **There are no approved therapies for the prevention of FNAIT. Standard care involves administering IVIG, a nonspecific immune suppressant, to mothers who have previously had an FNAIT-affected child. While IVIG can alleviate fetal and neonatal symptoms, it is associated with significant maternal side effects.**
- **We have previously demonstrated that the HPA-1a-specific monoclonal antibody RLYB212 induces rapid and complete elimination of HPA-1a+ platelets from circulation and prevents alloimmunization in non-pregnant mice, making it a potential prophylactic candidate to prevent FNAIT during pregnancy.**

RLYB212 eliminates of HPA-1a+ platelets



RLYB212 prevents alloimmunization



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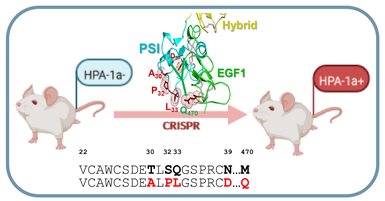
AIM

To evaluate the efficacy and safety of RLYB212 in preventing alloimmunization and FNAIT in pregnant mice

MOUSE MODEL

- **The HPA-1a/1b (also known as $PI^{A1/A2}$) dimorphism encodes a leucine (L) or a proline (P) residue, respectively, at position 33 of the integrin β3 subunit, and is responsible for ~80% of the cases of FNAIT.**
- **The murine integrin β3 subunit does not express the HPA-1a epitope.**
- **The epitope for HPA-1a-specific antibodies requires L_{33} but can also involve other amino acids in integrin β3.**
- **We used CRISPR/Cas9-mediated genome editing to create the HPA-1a alloepitope in mice by humanizing five amino acids ($A_{30}P_{32}L_{33}D_{39}Q_{470}$) in mouse integrin β3.**



METHODS

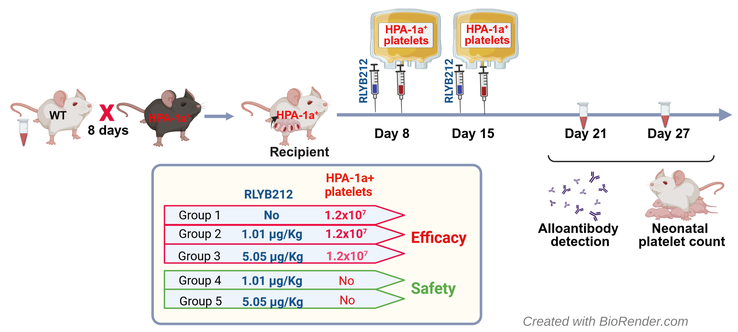


Figure 1 - Schematic illustration of RLYB212 prophylaxis for FNAIT in pregnant mice. *To evaluate the efficacy of RLYB212 in preventing alloimmunization and FNAIT, wild-type (WT) female BALB/c mice were mated with HPA-1a-homozygous male C57Bl/6 mice. Pregnant females carrying HPA-1a positive fetuses received prophylactic RLYB212 (via IV injection) on days 8 and 15 post-mating at two doses (1.01 or 5.05 µg/kg body weight), followed by transfusion of $1.2x10^7$ HPA-1a+ platelets (equivalent to a fetal-maternal hemorrhage (FMH) of ~30 ml in humans). Control mice received HPA-1a+ platelets but no RLYB212. To assess the safety of RLYB212 administration during pregnancy, pregnant females carrying HPA-1a positive fetuses received prophylactic RLYB212 (via IV injection) on days 8 and 15 post-mating without HPA-1a+ platelet transfusion. Pups' platelet counts were assessed within 48 hours of birth, and HPA-1a-reactive antibody levels were measured via flow cytometry.*

CONCLUSIONS

- **RLYB212 at doses of 1.01 or 5.05 µg/kg body weight prevents alloimmunization induced by transfusion of HPA-1a+ platelets in pregnant mice. RLYB212-treated, but not untreated, pregnant mice give birth to pups with normal platelet counts, indicating its efficacy in preventing FNAIT.**
- **RLYB212 treatment of pregnant mice, at either 1.01 or 5.05 µg/kg body weight, does not cause thrombocytopenia in HPA-1a+ pups, indicating its safety as a prophylactic treatment.**

Prophylactic administration of the HPA-1a-specific antibody RLYB212 to pregnant mice is both effective and safe in preventing maternal alloimmunization and FNAIT due to HPA-1a-incompatibility.

RESULTS

1. RLYB212 prevents maternal alloimmunization and FNAIT

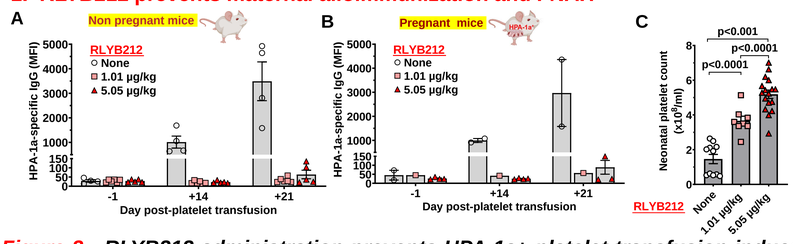


Figure 2 - RLYB212 administration prevents HPA-1a+ platelet transfusion-induced maternal alloimmunization and FNAIT. *(A, B) RLYB212 at doses of 1.01 or 5.05 µg/kg body weight effectively prevented alloimmunization to transfused HPA-1a-positive platelets in both non-pregnant (A) and pregnant (B) mice. HPA-1a-specific antibody levels were measured by flow cytometry. Results are presented as median fluorescence intensity (MFI) ± standard error of the mean. (C) HPA-1a-positive pups born to pregnant mice transfused with HPA-1a-positive platelets had significantly higher platelet counts if dams were treated vs. not treated with RLYB212, indicating prevention of FNAIT.*

2. RLYB212 treatment does not cause thrombocytopenia

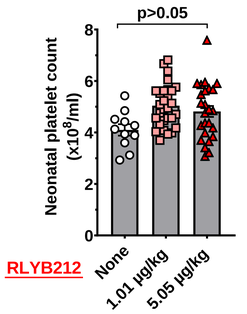


Figure 3 - RLYB212 administration during pregnancy does not cause thrombocytopenia in HPA-1a+ pups. *The safety of administering RLYB212 during pregnancy was assessed by monitoring pregnant mice given RLYB212 (1.01 or 5.05 µg/kg body weight) without HPA-1a+ platelet transfusion. Platelet counts in HPA-1a-positive pups born to pregnant mice treated with either dose of RLYB212 were not significantly different from those in pups born to pregnant mice not treated with RLYB212, indicating the safety for fetuses and neonates of RLYB212 administration during pregnancy.*

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