Insights Into Fetal & Neonatal Alloimmune Thrombocytopenia

Brian R. Curtis, Ph.D., MS, D(ABMLI), MLS(ASCP)SBB

Senior Director Diagnostic Hematology
Director Platelet & Neutrophil Immunology Lab
Senior Investigator Blood Research Institute
Versiti, Milwaukee, Wisconsin

versiti™

Disclosures

Name	Role	Disclosures
Brian Curtis, PhD, D(ABMLI), MLS(ASCP), SBB	Speaker	Consultant: Rallybio

Versiti Blood Research Institute

Human Platelet Alloantigens (HPA)

Antigens	Frequency (Whites)	Glycoprotein	Amino Acid Substitution	Gene / Variant
HPA -1a/a	72% a/a	GPIIIa/CD61	p.Leu33Pro	*ITGB3* / c.176T>C
HPA -1a/b	26% a/b			
HPA -1b/b	2% b/b			
HPA - 2a	99%	GP Ibα/CD42b	p.Thr145Met	*GP1BA* / c.482C>T
HPA - 2b	15%			
HPA - 3a	85%	GP IIb/CD41	p.Ile843Ser	*ITGA2B* / c.2621T>G
HPA - 3b	63%			
HPA - 4a	99.9%	GP IIIa/CD61	p.Arg143Gln	*ITGB3* / c.506G>A
HPA - 4b	<0.1%			
HPA - 5a/a	80%	GP Ia/CD49b	p.Glu505Lys	*ITGA2* / c.1600G>A
HPA - 5a/b	19%			
HPA - 5b/b	1%			
HPA-15 a/a	35%	CD109	p.Ser682Tyr	*CD109* / c.2108C>A
HPA-15 a/b	42%			
HPA-15 b/b	23%			

https://www.versiti.org/hpa

Human Platelet Alloantigens

HPA-6b	< 0.1%	GPIIIa / R489Q
HPA-7b	< 0.1%	GPIIIa / P407A
HPA-8b	< 0.1%	GPIIIa / R636C
HPA-9b	< 0.1%	GPIIb / V837M
HPA-10b	< 0.1%	GPIIIa / R62Q
HPA-11b	< 0.1%	GPIIIa / R633H
HPA-12b	< 0.1%	GPIbβ / G15E
HPA-13b	< 0.1%	GPIa / T799M
HPA-14b	< 0.1%	GPIIIa / K611de
HPA-16b	< 0.1%	GPIIIa / T140I
HPA-17b	< 0.1%	GPIIIa / T195M
HPA-18b	< 0.1%	GPIa / Q716H
HPA-19b	< 0.1%	GPIIIa / K137Q
HPA-20b	< 0.1%	GPIIb / T619M
HPA-21b	< 0.1%	GPIIIa / E628K
HPA-22b	< 0.1%	GPIIb / K164T
HPA-23b	< 0.1%	GPIIIa / R622W
HPA-24b	< 0.1%	GPIIIa / S472N
HPA-25b	< 0.1%	GPIa / T1087M
HPA-26b	< 0.1%	GPIIIa / K580N
HPA-27b	< 0.1%	GPIIb / L841M
HPA-28b	< 0.1%	GPIIb / V740L
HPA-29b	< 0.1%	GPIIIa / T33M†
HPA-30b	< 0.1%	GPIIb / Q806H
HPA-31b	< 0.1%	GPIX / P107L
HPA-32b	< 0.1%	GPIIIa / N148S
HPA-33b	< 0.1%	GPIX / D432G
HPA-34b	< 0.1%	GPIIIa / R91W
HPA-35b	< 0.1%	GPIIIa / R479H
NA	99.9 (Whites) 97%(Blacks) 96% (Asians)	CD36 (GPIV) (multiple variants)

★Antibodies against all platelet antigens can cause FNAIT!

https://www.versiti.org/hpa

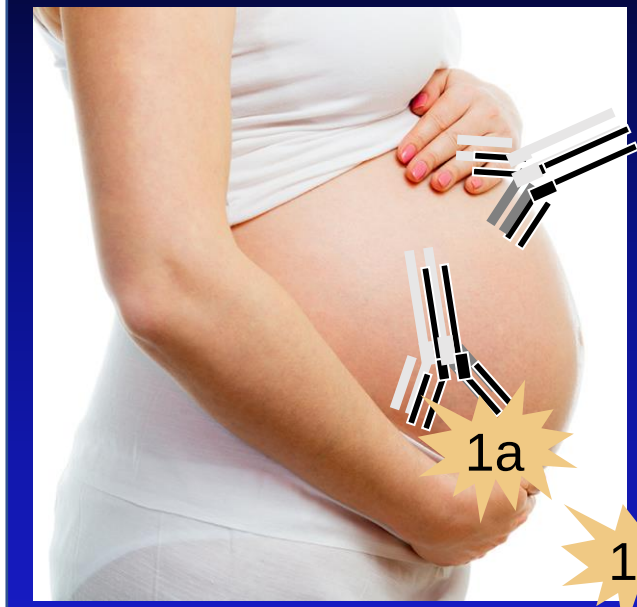
Fetal & Neonatal Alloimmune Thrombocytopenia

- Platelet counterpart of HDFN
- Occurs ~1:1000 births (~3.7M births x .001 = **3,700 cases/yr in USA**)
- Accounts for 3% of all neonatal thrombocytopenias and 27% of severe cases (platelets < 50K/µL)
- ~15% of cases occur in first pregnancies
- Most cases discovered after birth of the infant
- Majority of cases:

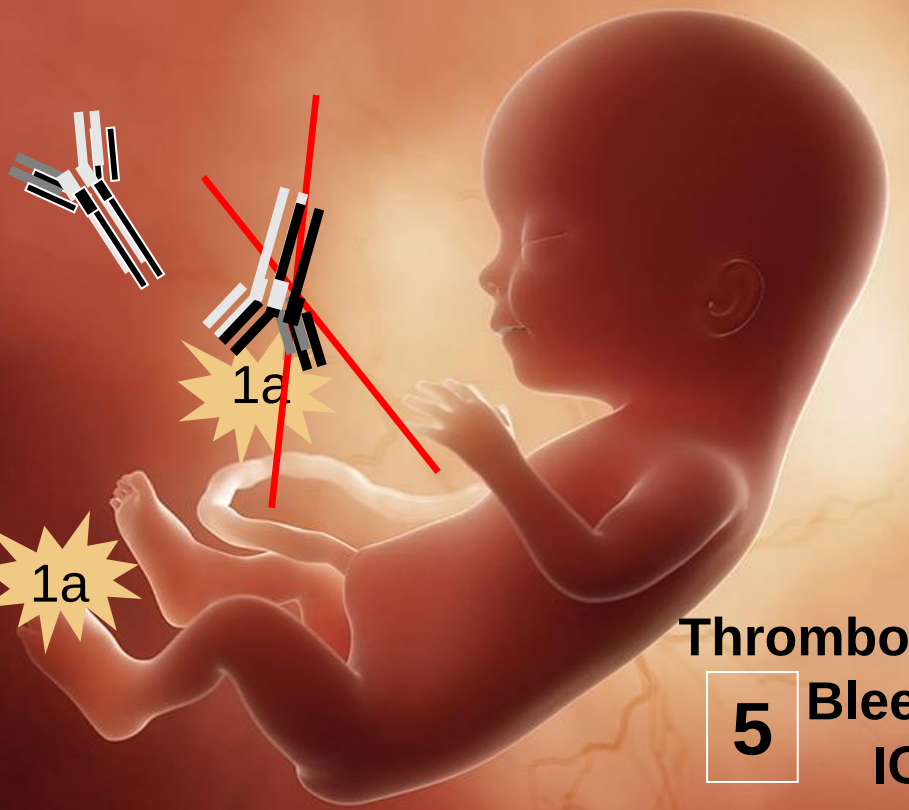
  HPA-1a (PI^{A1}) – Whites (>80%)
  HPA-4 - Japanese
  CD36 – Asians, Blacks, Middle East

FNAIT - Pathogenesis

1 **HPA-1b/b Mother (HPA-1a negative)**



2 **HPA-1a/1b Fetus (HPA-1a positive)**



4 **Maternal IgG HPA-1a antibodies**

1a 1a 1a 1a 1a

3 **Fetal cells HPA-1a platelets**

5 **Thrombocytopenia Bleeding ICH**

FNAIT - Clinical Features

- Infants' thrombocytopenia discovered within hours of birth – often resolves in 2-3 weeks, if untreated
- Maternal platelet count normal
- Petechiae, bruises, bleeding, GU/GI hemorrhages
- Intracranial hemorrhage (ICH) 11 in 10,000 (de Vos TW, et al. Lancet Haematol. 2023)
- Majority of ICH occurs antenatally, often before 28 weeks (Tiller H, et al. BMJ Open 2013)

HLA-DRB3*01:01 Association With Anti-HPA-1a Allo-Immunization

- ~30% of Whites are HLA-DRB3*01:01 positive
- DRB3*01:01 is associated with maternal HPA-1a antibody formation
- 0.6% of HPA-1b/1b women without this allele that deliver a HPA-1a+ infant make HPA-1a antibodies
- 12.7% of HPA-1b/1b women with this allele that deliver a HPA-1a+ infant make HPA-1a antibodies (**25x higher**)
- Is there utility in knowing maternal DRB3*01:01 type?

Transfusion 2019;59;1344–1352

When to Consider FNAIT

- Infant born with isolated thrombocytopenia (0 - 50K/µL), petechiae, bruising, bleeding (e.g., after circumcision)
- ICH that occurs in the absence of any apparent cause, e.g., anatomical defects or prematurity
- FNAIT confirmation infrequent for a fetus with isolated ICH or FBA
- Be vigilant with a pregnant woman serendipitously found to be HPA-1b/1b

Blood 2013; 122: 3660
Transfusion 2018; 58:223-31
Fetal Diagn Ther 2017; 42: 35-41

Severe FNAIT

Severely affected infant photo

Specificities in 1163 Serologically Proven FNAIT Cases (1990-2002)

(Davoren et al. *Transfusion* 44:1220-25, 2004)

Antibody Specificity	Number	Per Cent
HPA-1a	922	79.4
HPA-5b	109	9.3
HPA-1b	43	3.7
HPA-3a	20	1.7
HPA-3b	9	0.8
HPA-5a	12	1.0
HPA-4a	2	0.2
HPA-2b	1	0.1
GPIV	5	0.5
Total	**1123**	**96.7**

Specificities in 1163 Serologically Proven FNAIT Cases (1990-2002)

(Davoren et al. *Transfusion* 44:1220-25, 2004)

Antibody Specificity	Number	Per Cent
HPA-4b (Penb)	2	0.2
HPA-6wb (Ca/Tu)	2	0.2
HPA-1a + -5b	22	1.5
HPA-1b + 5b	5	0.5
HPA-1b + 3a	4	0.4
HPA-1b + 5a	1	0.1
HPA-1a + 3a + 2b	1	0.1
HPA-1b + 3a + 5a	1	0.1
HPA-3a + 5a	1	0.1
HPA-3b + 2b	1	0.1
Total	**40**	**3.3**

Optimal Lab Workup for FNAIT

- Tests of maternal serum for antibodies against HPA
  - Intact platelet assay (flow cytometry) AND antigen-capture assay like the MACE, MAIPA, PABA
- ★Cross-match of maternal serum and father's platelets
  - Detects antibodies to low frequency and new HPA
- HPA genotype mother and father or infant
  - HPA-1, -2, -3, -4, -5, -15, ideally also -6, -9
- Additional testing as required
  - Gene sequencing, ABO type parents, CD36 typing, HLA?

J Thromb Haemost. 2018 Dec;16(12):2526-2529

Importance of Performing X-match with Father's Platelets

PABA Results:	**Platelet Targets and HPA Phenotypes**					
Maternal serum	T1	T2	T3	T4	T5	
	HPA-1aa 2aa	HPA-1bb 2ab	HPA-1ab 2bb	HPA-1aa 2aa	HPA-1bb 2aa	
Glycoprotein Bead	3aa 4aa 5aa	3bb 4aa 5bb	3ab 4aa 5aa	3bb 4aa 5ab	3aa 4aa 5aa	RESULTS
HLA	3	2	3	3	3	Negative
GPIa/IIa	2	3	3	2	2	Negative
GPIb/IX	2	3	2	2	3	Negative
GPIIb/IIIa	3	2	2	2	2	Negative
GPIV	2	3	2	2	2	Negative

≥ 5.0 = positive results

PABA X-match		
Glycoprotein Bead	**Fathers Platelets**	RESULTS
HLA	3	Negative
GPIa/IIa	2	Negative
GPIb/IX	2	Negative
GPIIb/IIIa	**130**	**Positive**
GPIV	2	Negative

← Low Frequency HPA-32b

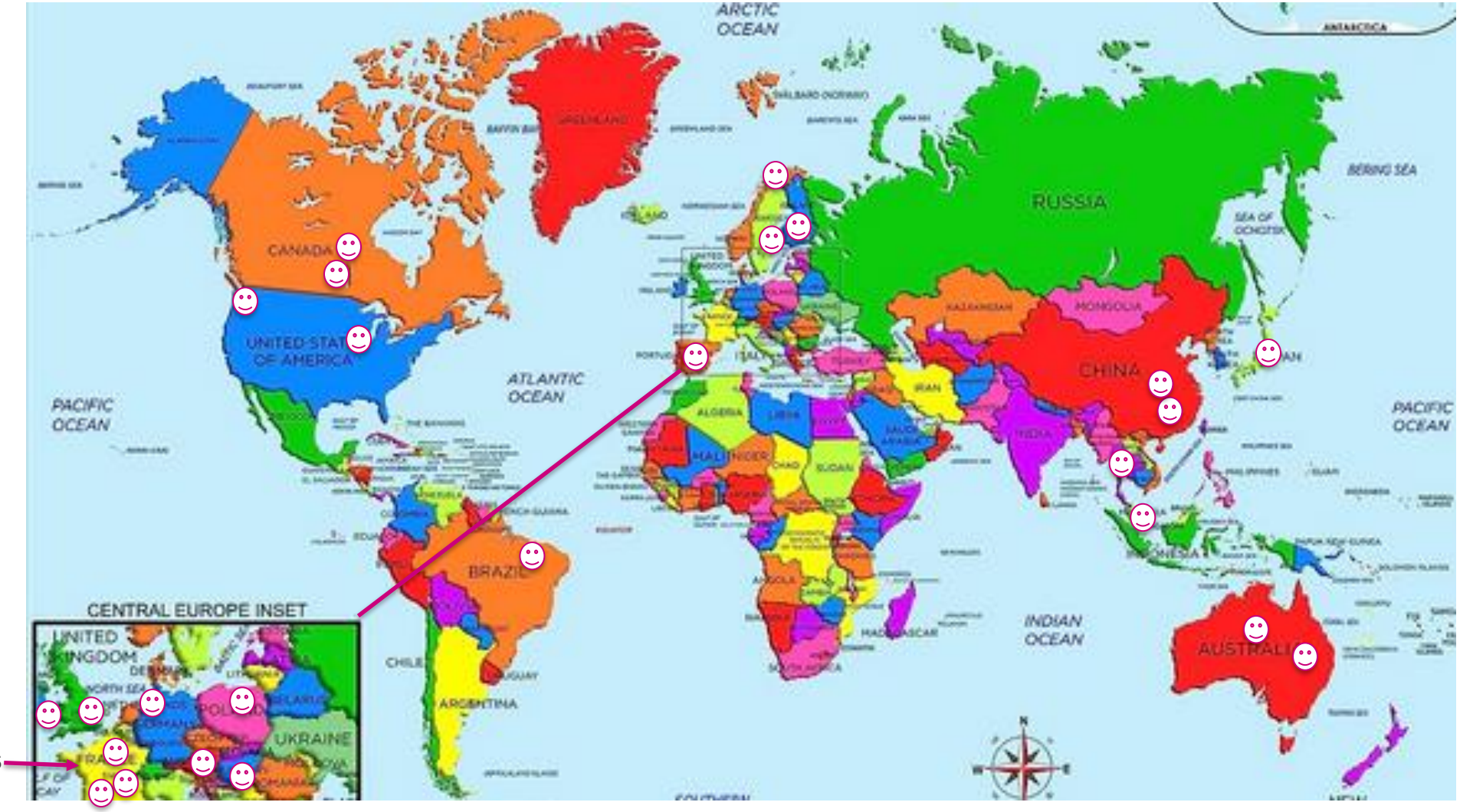
Distribution of PIWP Labs

30 Labs

19 Countries

Platelet Antibody Labs Across the World

Australia (2)
Austria (1)
Brasil (1)
Canada (2)
China (2)
Finland (1)
France (3)
Germany (4)
Great Britain (1)
Israel (1)
Japan (1)
Netherlands (1)
Norway (1)
Malaysia (1)
Slovenia (1)
Spain (1)
Sweden (1)
Thailand (1)
USA (2)
Wales (1)



FNAIT - Treatment

Treatment (newborn):

- Observation, platelet count monitoring
- HPA-negative platelet transfusion(s) post delivery +/-IVIgG
- Transfuse random platelets if HPA-matched not available
- Washed maternal platelets can be used too

FNAIT Case No.1650

- 26 y.o. G1P1, group O female and group O father
- Uncomplicated, full-term delivery of 8.2 lb male infant
- Bled profusely after circumcision D2 of life
- 5K plt/µl (normal = 150K -450K/µL)
- 2 CNS bleeds on head scan, 1 antenatal
- Random platelet transfusion failed to sustain platelet count
- Transfused washed maternal platelets, count increase to 50K/µl

FNAIT Case No.1650

Maternal serum IgG reactivity:

GP	T1	T2	T3	T4	T5	
Bead	HPA-1aa 2aa 3aa	1bb 2ab 3bb	1ab 2bb 3ab	1aa 2aa 3bb	1bb 2aa 3aa	**Result**
	4aa 5aa	4aa 5bb	4aa 5aa	4aa 5ab	4aa 5aa	
GPIIb/IIIa	**242**	3	**219**	**224**	1	**HPA-1a +**
GPIb/IX	2	1	2	4	1	Neg
GPIa/IIa	3	3	4	2	2	Neg
CD36	2	3	3	3	1	Neg
HLA	8	6	5	12	5	HLA +

Positive ≥ 5

Mother Genotype: HPA-1b/1b, 2a/2a, 3a/3a, 4a/4a, 5a/5a, 6a/6a, 9a/9a, 15a/b

Father Genotype: HPA-1a/1b, 2a/2a, 3a/3a, 4a/4a, 5a/5b, 6a/6a, 9a/9a, 15b/15b

FNAIT Case No.1650

Future Pregnancies:

- Father heterozygous - HPA-1a/b
- 50% risk of future fetus being affected by FNAIT
- Genetic counseling, prenatal genotype future fetus

Mother: HPA-1b/b
Father: HPA-1a/b

prenatal genotype →

50%

Fetus: HPA-1b/b - No treatment
Fetus: HPA-1a/b - IVIgG

Father: HPA-1a/a

~~prenatal genotype~~ →

100%

Fetus: HPA-1a/b - IVIgG

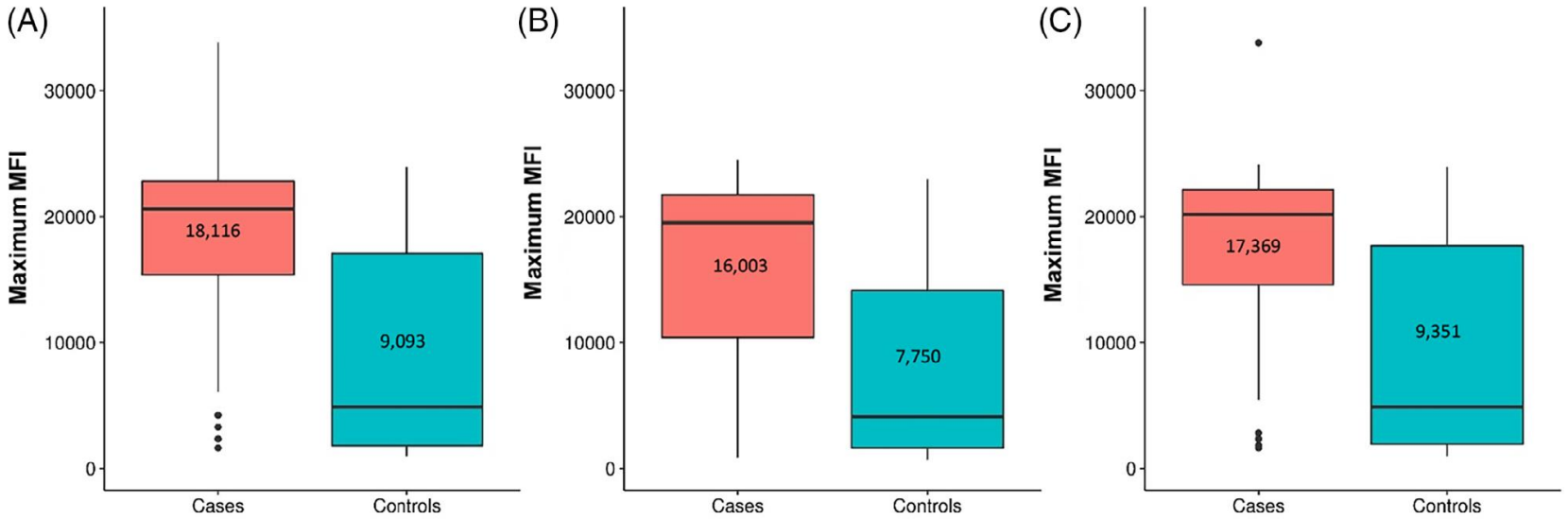
How to Manage a Subsequent Pregnancy When No Maternal HPA Antibodies Detected

- Screen maternal serum for HPA antibodies at 12, 24, 32 weeks gestation (serial monitoring)
- If HPA antibodies detected – consider maternal antenatal IVIgG
- If no HPA antibodies detected – No treatment
- What about maternal class I HLA antibodies?

LD Pacheco, et al. OBSTETRICS & GYNECOLOGY 2011; vol. 118, No. 5, 1157

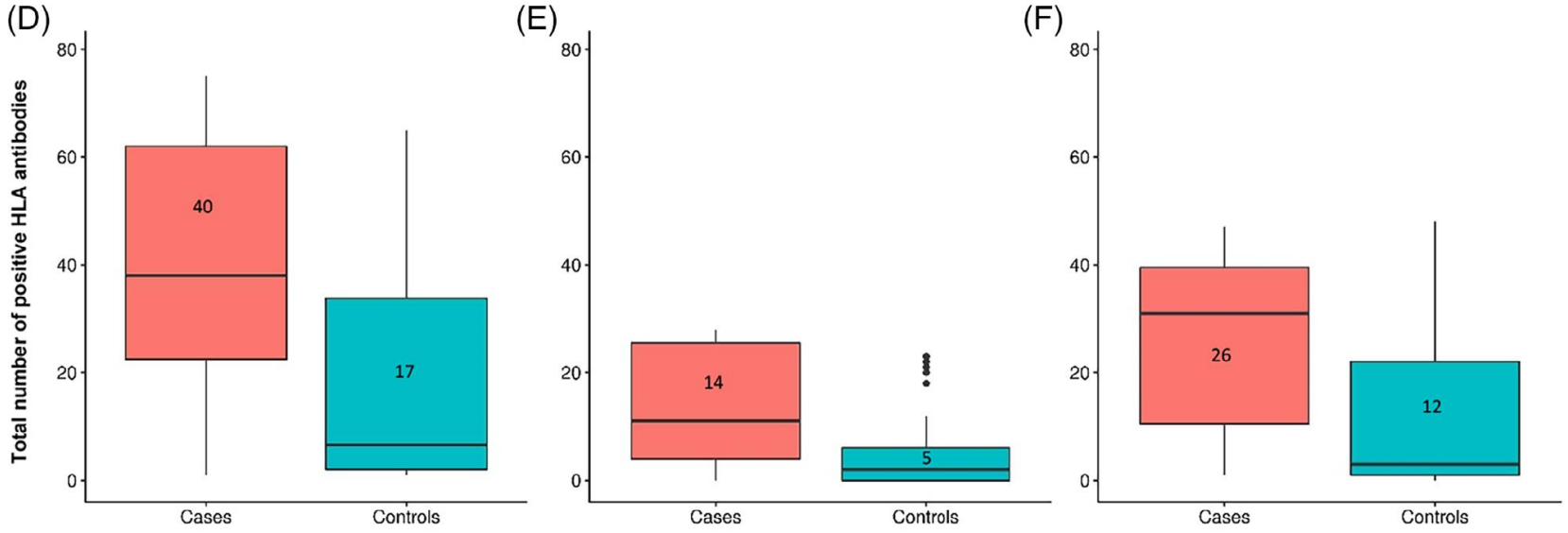
Maternal HLA Antibody Strength, Specificity

HLA antibody strength



Red = Cases
Blue = Controls

HLA antibody specificity



Z Colvin, …BR Curtis. *Transfusion* 2023;63(6).

FNAIT Management

Antenatal IVIgG:

- 1 g/kg/week, 98.7% effective against ICH
- Expensive, side-effects, not 100% effective

Steroids:

- 0.5 g/kg/week
- oligohydraminous, diabetes, questionable effectiveness

PUBS and intra-uterine platelet transfusions:

- NO LONGER RECOMMENDED!

FNAIT - Recent Developments

High vs. Low Risk for FNAIT

TABLE

Neonatal outcomes stratified on maternal antenatal intravenous immunoglobulins treatment status in subsequent pregnancies

FNAIT Risk group	§No antenatal IVIg (Norwegian cohort) Pregnancies n	Neonates with ICH n (%, 95% CI)	Antenatal IVIg (control group) Pregnancies n	Neonates with ICH n (%, 95% CI)	*P* value[a]
*Low-risk	64	0 (0, 0.0–5.7)	313	2 (0.6, 0.2–2.3)	1.00
High-risk	7	2 (29, 8.2–64.1)	90	5 (5.6, 2.4–12.4)	.08

Low-risk indicates pregnancies where a previous child had FNAIT without ICH; High-risk indicates pregnancies where an older sibling had ICH because of FNAIT.

CI, confidence interval; *FNAIT*, fetal and neonatal alloimmune thrombocytopenia; *ICH*, intracranial hemorrhage; *IVIg*, intravenous immunoglobulin; *n*, numbers.

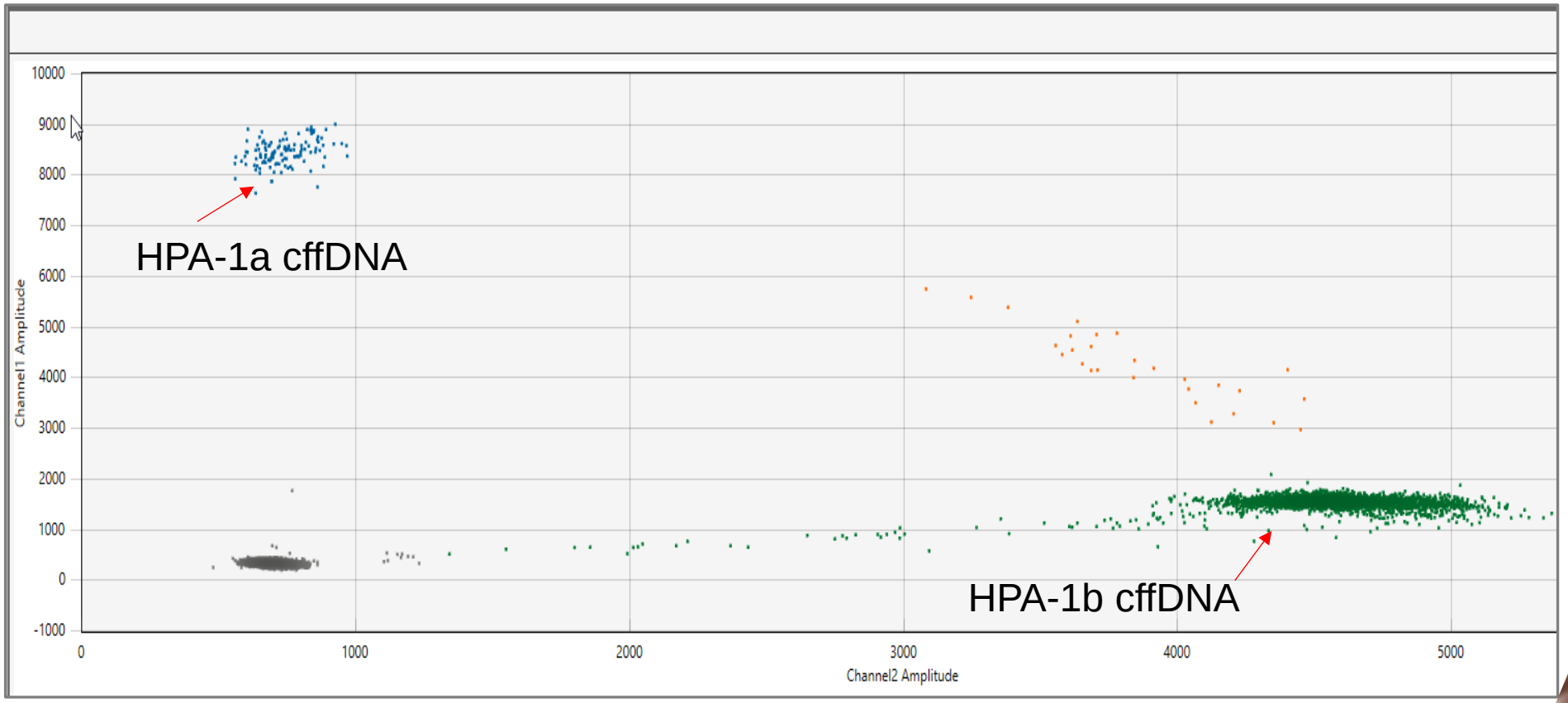
[a] *P* value was calculated by Fisher exact test.

Ernstsen et al. Antenatal intravenous immunoglobulin in pregnancies at risk of fetal and neonatal alloimmune thrombocytopenia. Am J Obstet Gynecol 2022.

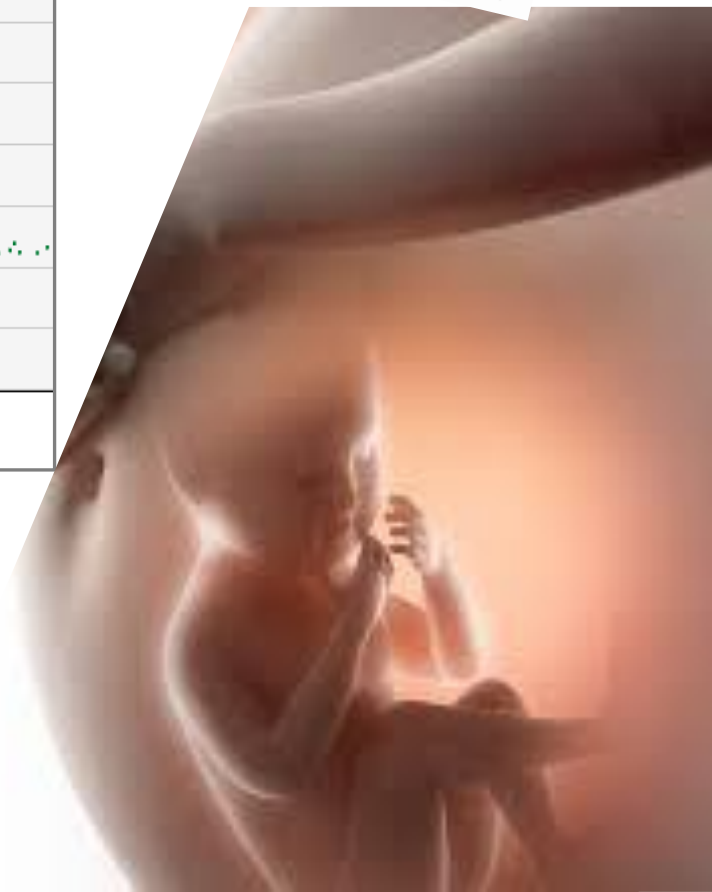
*Low risk < 3 IU/mL anti-HPA-1a

§Almost all delivered by C-section 1 - 2 weeks before term, prompt transfusion of compatible platelets to newborn

HPA-1Genotyping Cell-free Fetal DNA

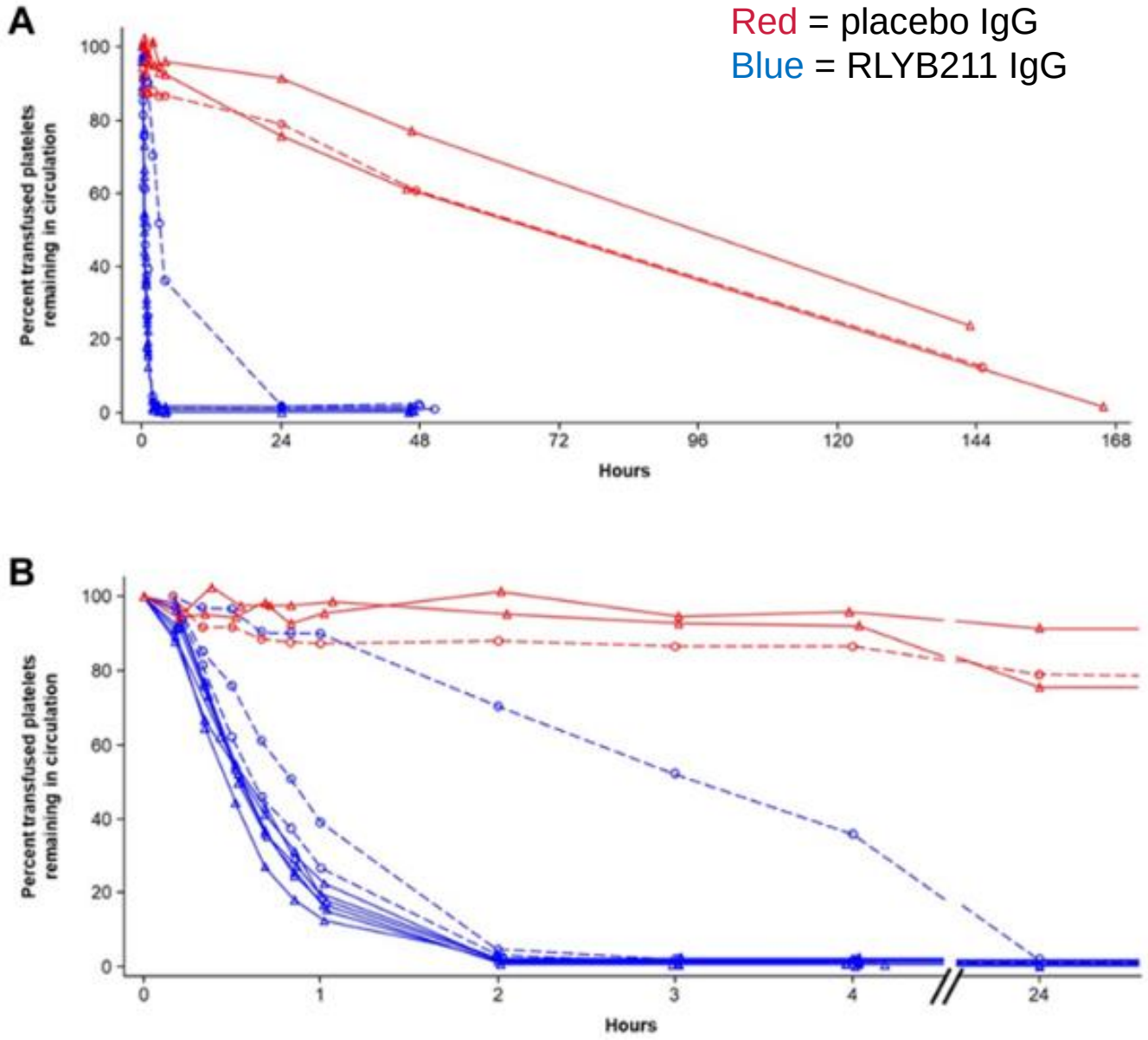


Maternal whole blood



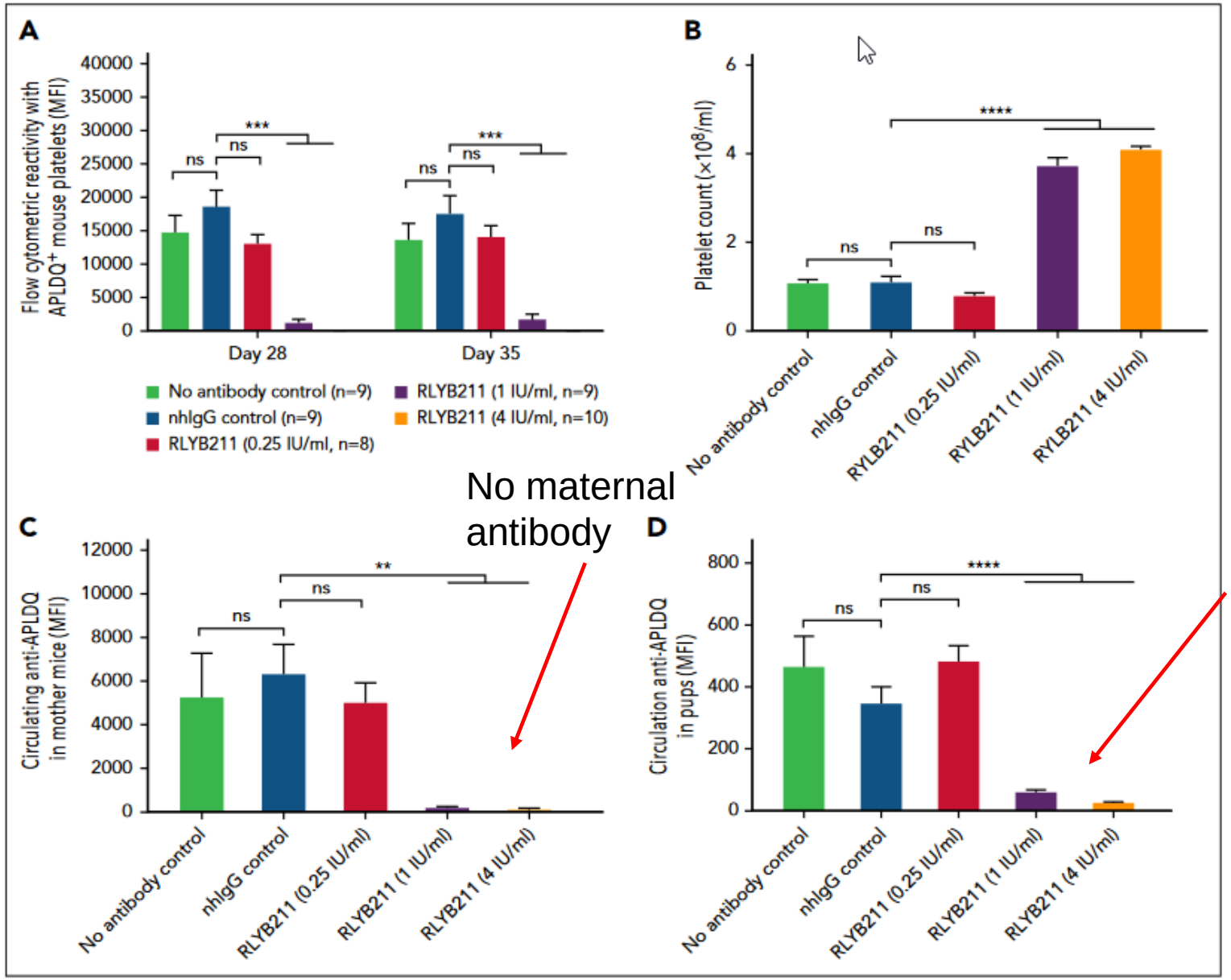
HPA-1a Antibody Prophylactic

- 12 healthy males – HPA-1b/1b, HLA-A2 neg (3 PL and 9 RLYB211)
- 1000 IU antibody
- HPA-1a/b, $A2^{+}$ plts transfused, ~30 mL fetal blood
- ≥90% reduction in $t^{1/2}$ of HPA-1a–positive platelets relative to placebo
- No participants developed HPA-1a antibodies at 12 or 24 weeks
- Two adverse events possibly related to treatment, both in RLYB211–treated participants



J Thromb Haemost 2023;21:838–849

RLYB211 Prevents FNAIT in Mouse Model



No maternal antibody

No antibody in pups

H Zhi, et al. *Blood* 2022;140(20)

RLYB212 Prevents Alloimmunization & FNAIT in Mouse Model

1185
Program: Oral and Poster ASH Abstracts
Session: 311. Poster I

Saturday, December 7, 2024, 5:30 PM-7:30 PM

Huiying Zhi, *Douglas Sheridan, PhD, Peter J. Newman, PhD and Debra K. Newman, PhD*

Figure 3

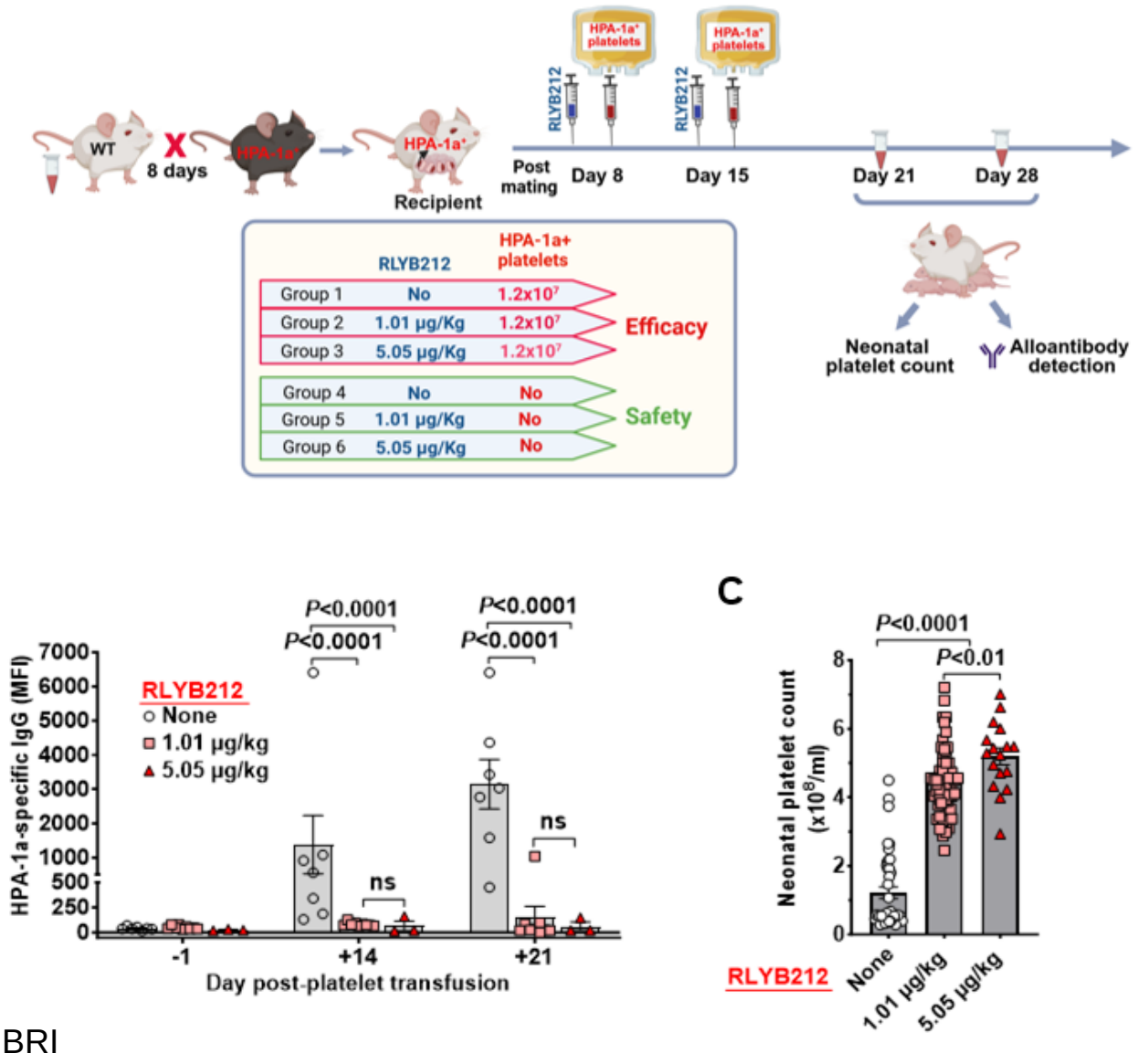


Figure kindly provided by Dr. D. Newman, Versiti BRI

HPA-1a Antibody Prophylactic

Study Sponsor - Rallybio Ipa LLC(RLBY212 anti-HPA-1a monoclonals)

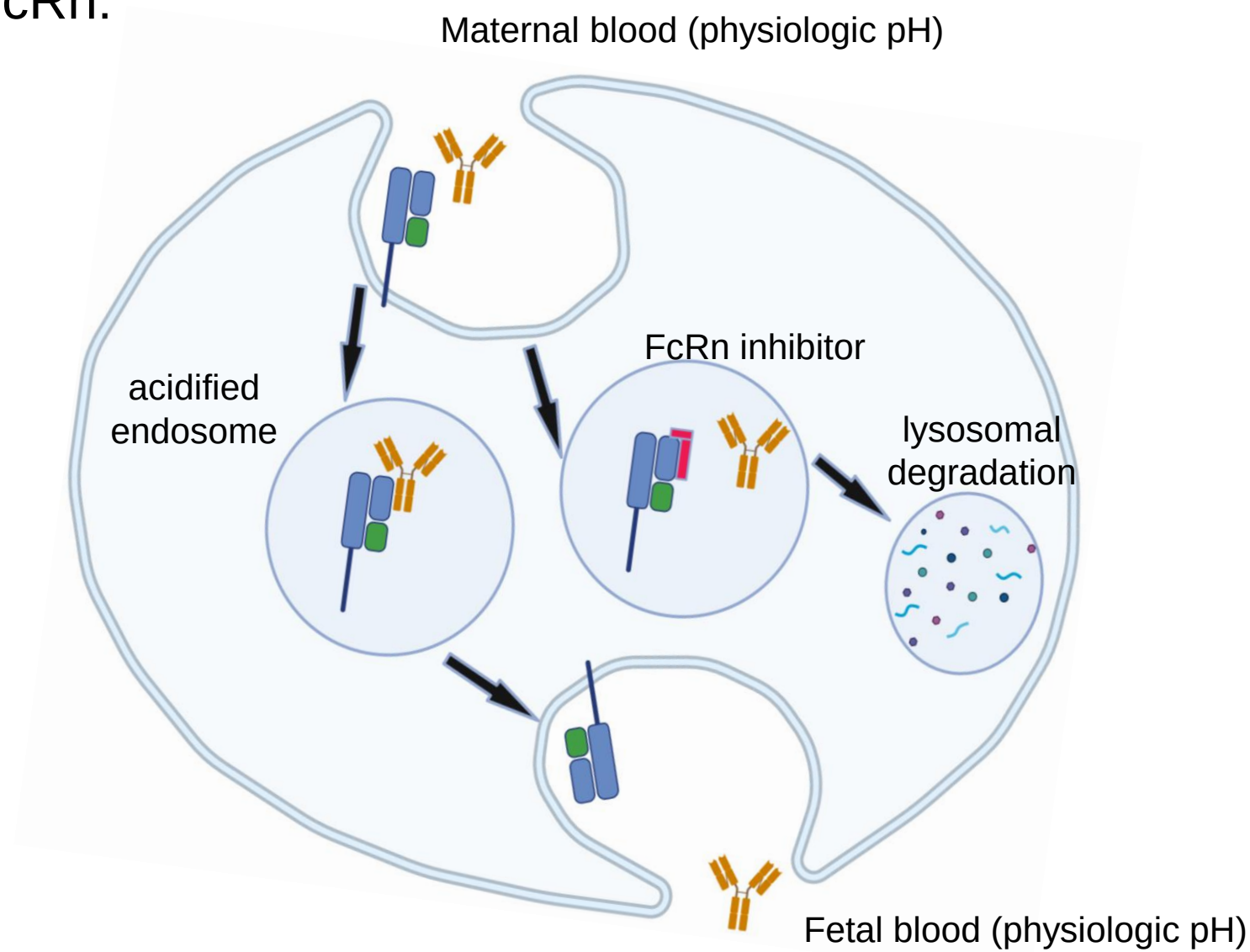
- Ongoing prospective, non-interventional, multinational **FNAIT Natural History Study** to screen 30,000 expectant mothers for higher FNAIT risk. Gestation week 10 to 14 prenatal visit. Screened for HPA-1a/1b genotype, HLA-DRB3*01:01 genotype, HPA-1a antibodies, fetal HPA-1 genotype

- Phase 2 Study on the Pharmacokinetics and Safety of RLYB212 in Pregnant Women at Higher Risk for HPA-1a Alloimmunization.

https://rallybio.com/pipeline/rlyb212/

Blocking FcRn Maternal-Fetal Transport of HPA Antibodies to Reduce the Risk of FNAIT

Transport of human IgG by FcRn:



Studies of Nipocalimab in Reducing the Risk of Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)

Study Sponsor - Janssen Research & Development, LLC

- **FREESIA-1:** Double-blind, randomized, placebo-controlled, multicenter study. History >= 1 prior pregnancy with FNAIT none affected by ICH or other severe hemorrhage. Current pregnancy with presence of maternal anti-HPA-1a and HPA-1a^{+} fetus.

- **FRESSIA-3:** Open-label, multicenter, randomized, phase 3 study enrolling HPA-1a and/or HPA-5b, alloimmunized maternal participants with an HPA-1a or HPA-5b-positive fetus and a prior FNAIT. Randomized to weekly intravenous nipocalimab or IVIG with/without prednisone.

https://clinicaltrials.gov/study/NCT06449651?cond=FNAIT&rank=2
https://fetalmedicine.org/abstracts/2024/var/pdf/abstracts/2024/06256.pdf

Critical Tools for Further Advancements

- Develop and validate test for HPA-1a antibody levels
- Establish HPA-1a antibody levels that predict high vs. low risk
- Validate cell-free fetal DNA genotyping tests for HPA