

# Effect of donor, component, and recipient characteristics on hemoglobin increments following red blood cell transfusion

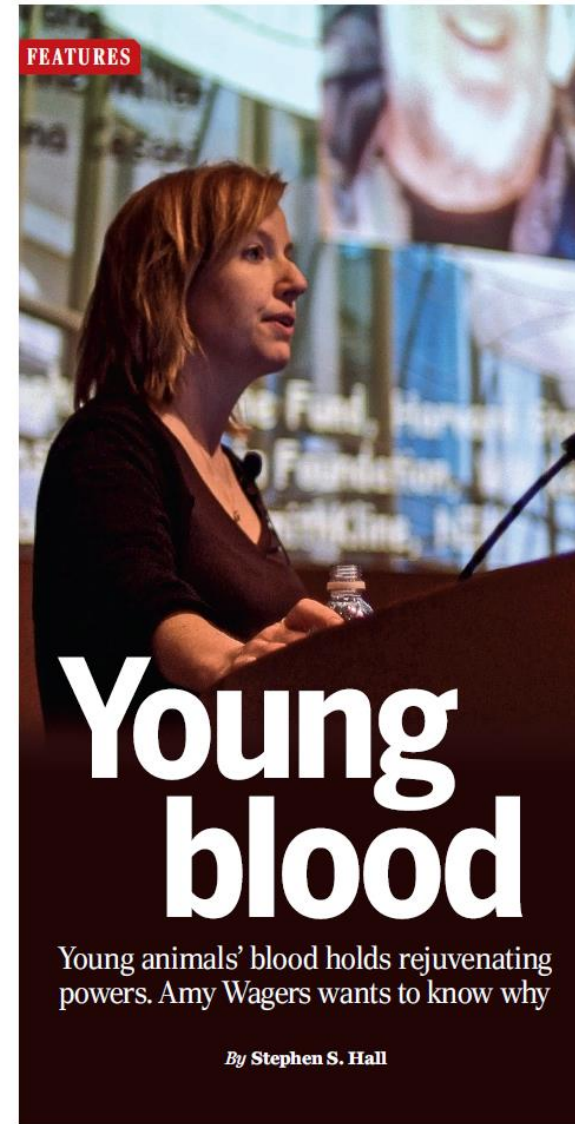
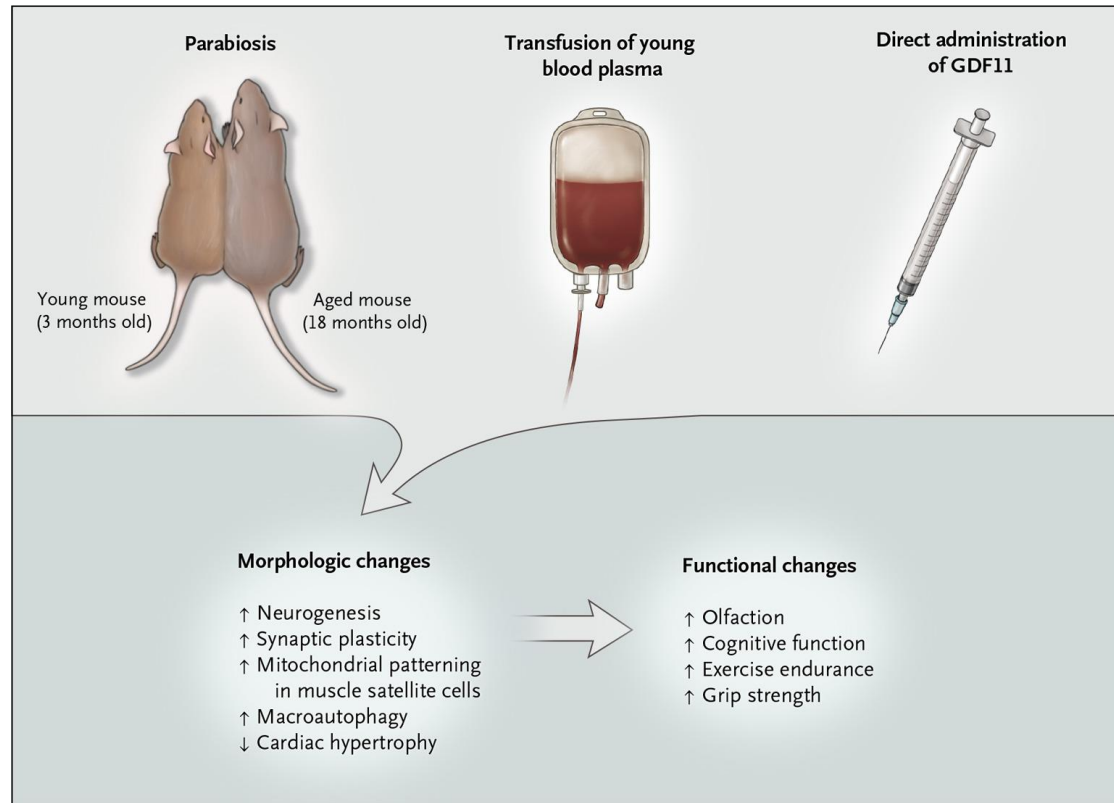
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Vitalant Research Institute

Kaiser Permanente Division of Research

## Background: RBC effectiveness studies

- RBCs are a complex biological product with variation between units
- Known differences in stress-induced hemolysis at end of RBC storage
- Prior studies have focused on the impact of prolonged RBC storage on morbidity and mortality
- Recent studies are using “big data” approaches
  - Evaluate blood donor, component, and recipient variables



**A**my Wagers was about halfway through a 90-minute talk to a group of Boston-area science teachers last July when she showed her Arnold Schwarzenegger slides. One picture depicted the former governor of California as a buff young bodybuilder; the other showed a more recent, flabbier, and stooped version of the Terminator playing tennis. If a picture is worth a thousand words, the slide served as a two-volume treatise illustrating the point that Wagers, a Harvard University stem cell scientist, wanted to make: As humans age, our muscles fail to maintain and regenerate as they once did.

"I'm not saying aging is a disease," Wagers hastened to add, once her audience had stopped laughing. "But it is associated with increased incidence of particular types of diseases."

Wagers's quiet voice barely carried over an aspiring aquarium at the side of the science lab at Dover-Sherborn High School. But the 30 or so educators gathered at the Summer Science Institute avidly followed the narrative of her recent work—especially when Wagers moved on to her "fountain of youth" slides.

In a series of experiments that have captivated both the field of regenerative medicine and its many lay spectators, Wagers and a diverse army of collaborators have shown that when the blood of a young mouse circulates through the murine equivalent of an old geezer, startling physiological changes occur. Many of the trademark depredations of old age—withering muscles; stiff, oversized hearts; cognitive decline; and even the fraying of the myelin coating that insulates nerve fibers—are slowed, repaired, or even reversed.

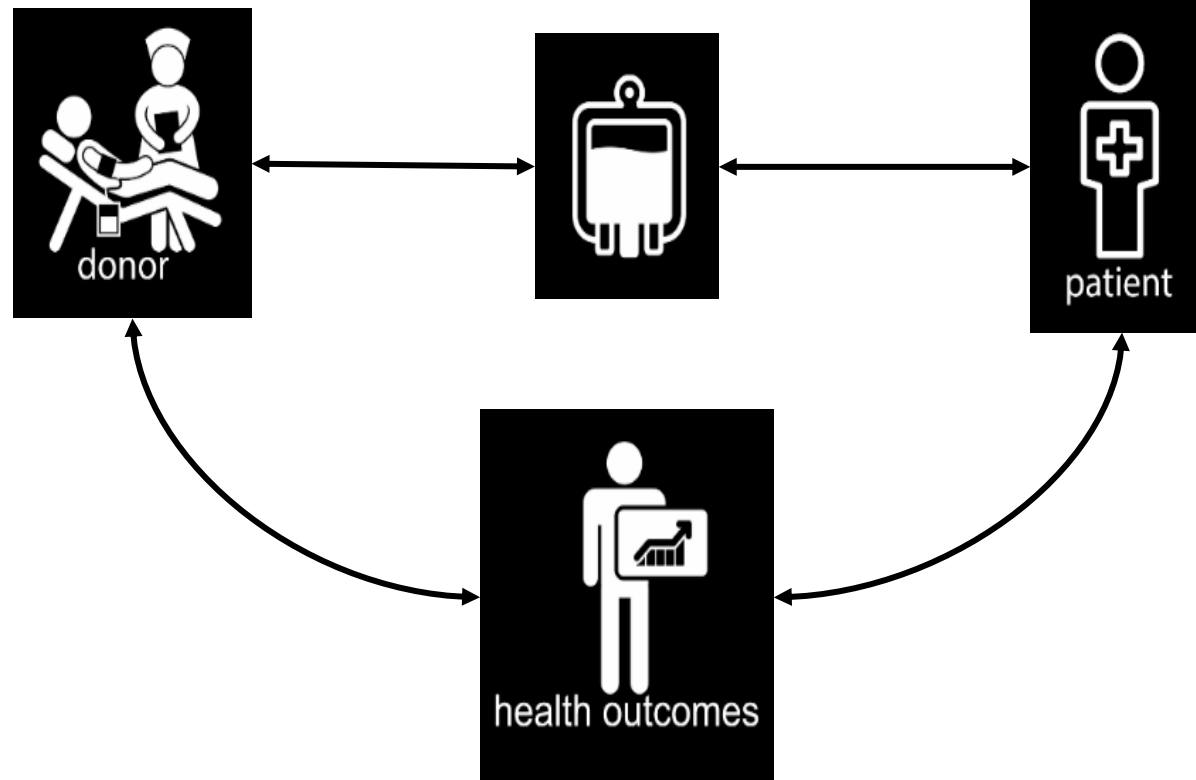
"We became convinced that there was something in the blood" responsible for the dramatic effects, Wagers told the teachers. Indeed, after a difficult search, she and colleagues have recently isolated a molecule from "young blood," growth differentiation factor 11 (GDF11), that appears to rejuvenate the architecture of the heart, the vasculature of the brain, and the bulk of skeletal muscle—at least in animals. As Wagers told the group, "GDF11, which is this 'fountain of youth' kind of factor for the heart, is also a fountain-of-youth factor for the skeletal muscle and for the brain. It is generally a good protein for rejuvenation."

"Rejuvenation" has always been a loaded crossover term in biology. Quickly raising her hand, one young high school science teacher from Walpole could barely spit out the question on everyone's mind. "You have to wonder, given what you're seeing ... I mean,

# Donor-Component-Recipient Linkages

Collection, manufacturing  
and processing data

Demographics,  
social behavior,  
medical history  
on each donation

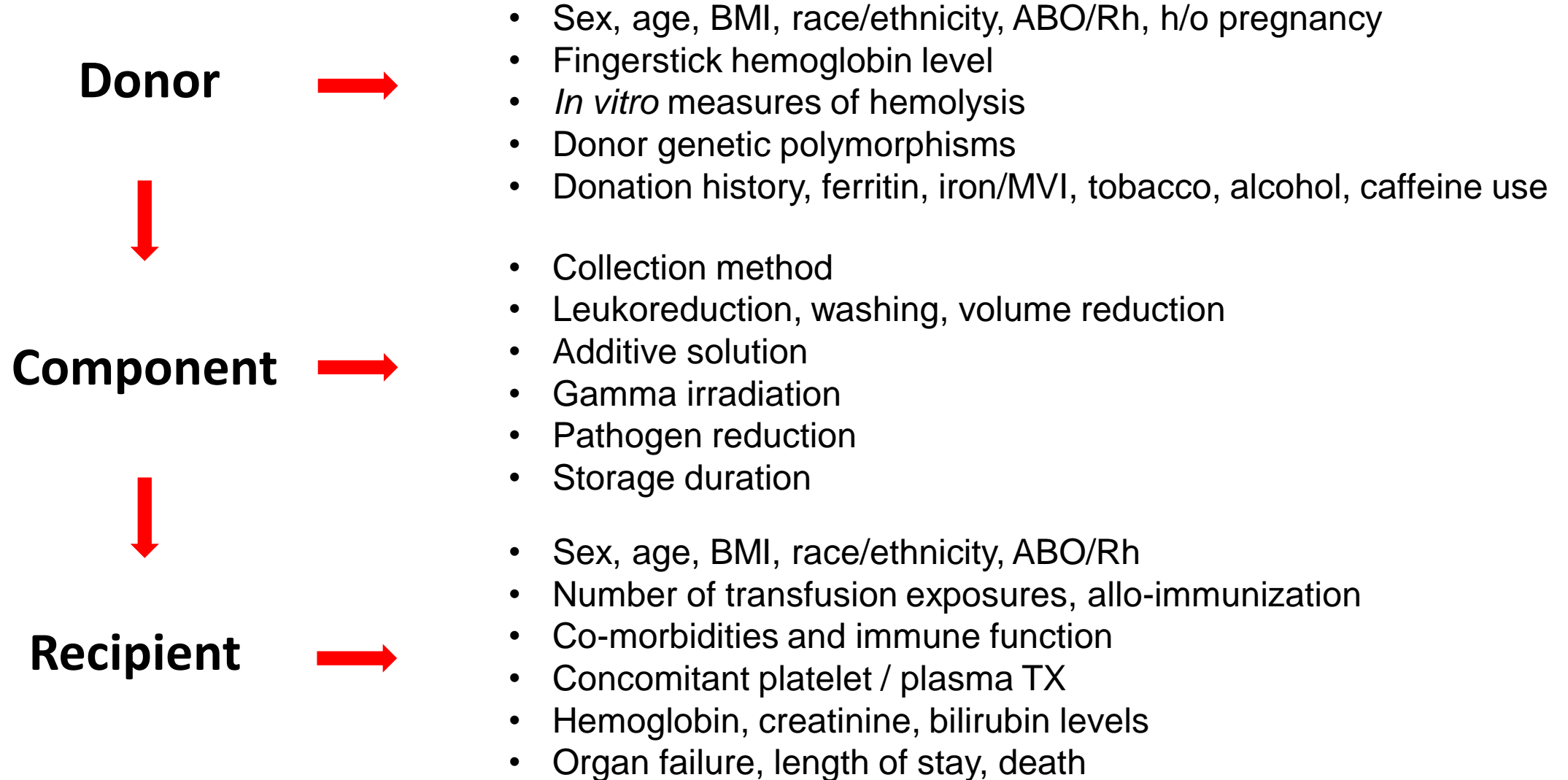


Electronic  
health records,  
registries,  
claims data

# Blood donor-component-recipient databases

- Canada
  - TRUST (Hamilton/McMaster)
  - Ottawa (OHRI)
- Sweden/Denmark
  - SCANDAT
  - Dutch Blood Donor Study (DBDS)
- Netherlands
  - DTD
- United States
  - REDS-III/IV (Recipient Epidemiology Donor Evaluation Study)
  - Kaiser Permanente Northern California

# Donor-Component-Recipient Linkages



Original Investigation

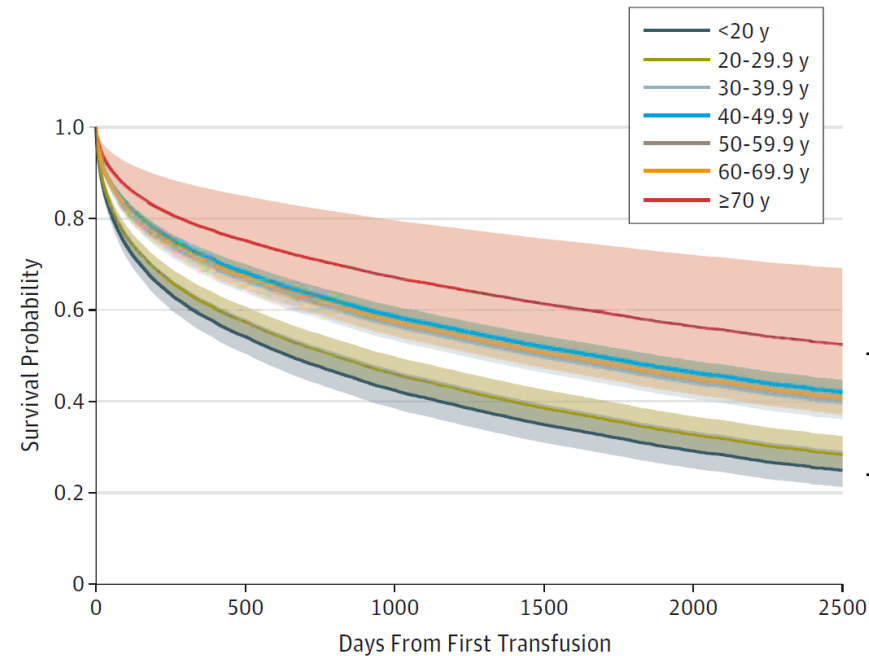
# Association of Blood Donor Age and Sex With Recipient Survival After Red Blood Cell Transfusion

Michaël Chassé, MD, PhD, FRCPC; Alan Tinmouth, MD, MSc, FRCPC; Shane W. English, MD, MSc, FRCPC; Jason P. Acker, MBA, PhD; Kumanan Wilson, MD, FRCPC; Greg Knoll, MD, MSc, FRCPC; Nadine Shehata, MD, MSc, FRCPC; Carl van Walraven, MD, MSc, FRCPC; Alan J. Forster, MD, MSc, FRCPC; Timothy Ramsay, PhD; Lauralyn A. McIntyre, MD, MSc, FRCPC; Dean A. Fergusson, MHA, PhD

**IMPORTANCE** While red blood cells (RBCs) are administered to improve oxygen delivery and patient outcomes, they also have been associated with potential harm. Unlike solid organ transplantation, the clinical consequences of donor characteristics on recipients have not been evaluated in transfusion medicine.

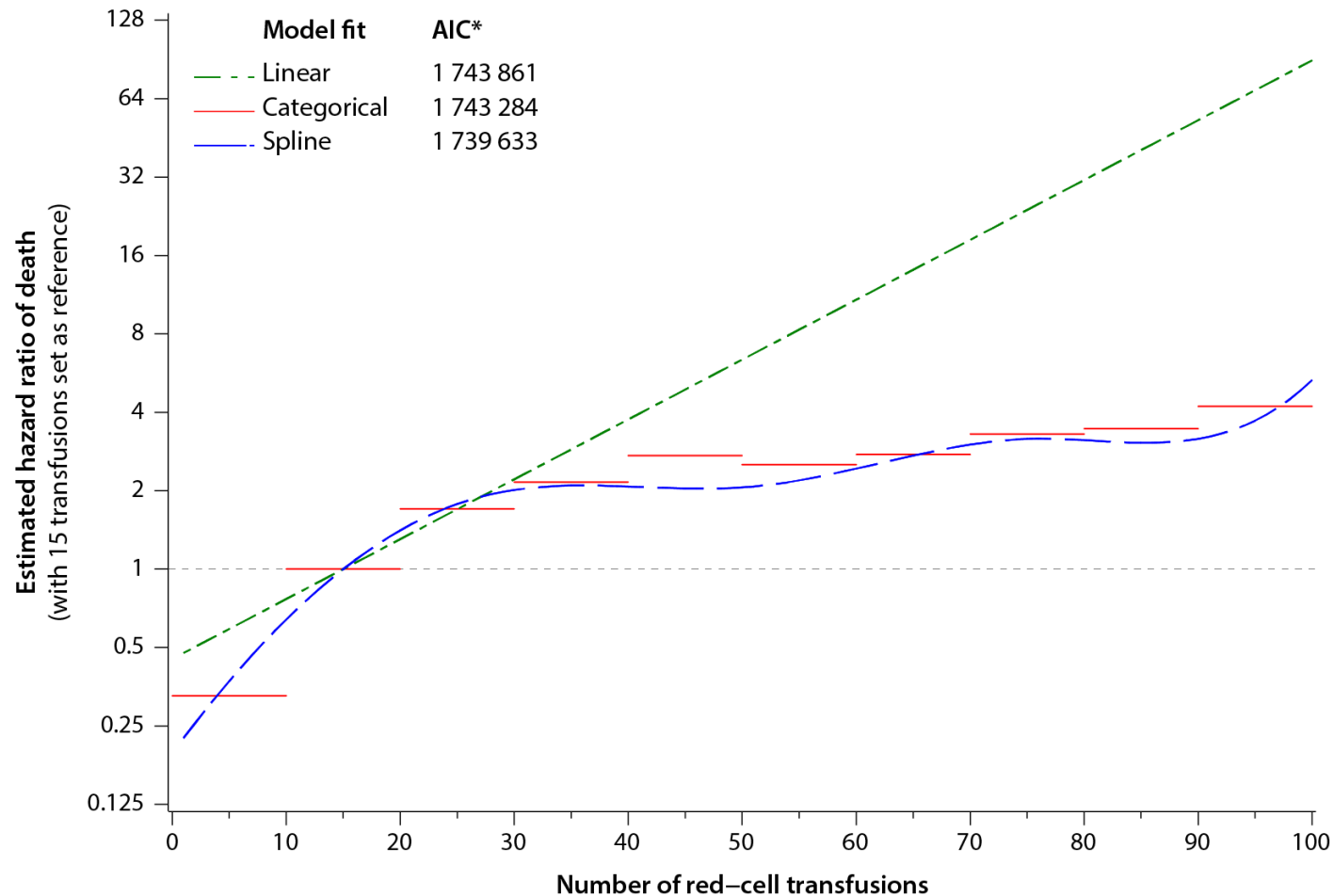
← Invited Commentary

+ Supplemental content at [jamainternalmedicine.com](http://jamainternalmedicine.com)



>25% absolute mortality difference

# # of RBCs & mortality: non-linear association





# Donor age & patient mortality: adjustment for number of RBC transfusions

Donor age	Number of RBC units (%)	Log-linear adjustment	Restricted cubic splines
		<i>Hazard ratio (95% CI)</i>	
<20 years	126 847(1.9)	1.04 (1.03-1.04)	1.01 (1.00-1.01)
20-29 years	1 104 248(16.3)	1.02 (1.02-1.02)	0.99 (0.99-1.00)
30-39 years	1 464 872(21.6)	1.00 (1.00-1.00)	0.99 (0.99-1.00)
40-49 years	1 889 084(27.9)	1.00 (ref)	1.00 (ref)
50-59 years	1 600 320(23.6)	1.00 (1.00-1.00)	1.00 (1.00-1.00)
60-69 years	578 194(8.5)	1.00 (1.00-1.01)	1.01 (1.01-1.02)
≥70 years	3 238(0.0)	0.85 (0.83-0.87)	0.96 (0.91-1.01)

# Associations between donor sex, prior pregnancy & mortality

Table 3. Mortality Hazard Ratio of Male Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female Ever-Pregnant Donors vs Male Donors in the No-Donor-Mixture, Single-Transfusion, and Full Cohorts, Stratified by Patient Age<sup>a</sup>

Donor Category	No. of Deaths Among Recipients/No. of Recipients, by Recipient Age				P Value for Interaction <sup>b</sup>
	0-17 y	18-50 y	51-70 y	≥71 y	
<b>No-Donor-Mixture Cohort<sup>c</sup></b>					
Male (reference)	107/2251	84/1170	598/4292	933/4499	
Ever-pregnant female	17/305	10/126	47/483	77/543	
HR (95% CI) <sup>d</sup>	1.63 (1.02-2.61)	1.50 (0.98-2.30)	1.10 (0.91-1.33)	1.06 (0.90-1.26)	.10
P value	.04	.06	.31	.47	
<b>Single-Transfusion Cohort<sup>e</sup></b>					
Male (reference)	53/1993	16/411	129/1686	236/2099	
Ever-pregnant female	17/294	7/93	23/363	51/440	
HR (95% CI) <sup>d</sup>	2.84 (1.58-5.12)	2.29 (0.89-5.93)	0.79 (0.50-1.25)	1.06 (0.78-1.46)	<.001
P value	.001	.09	.32	.71	
<b>Full Cohort</b>					
Male (reference)	124/2421	146/1565	922/5570	1346/5748	
Ever-pregnant female	17/224	10/84	47/347	77/379	
HR (95% CI) <sup>d</sup>	1.18 (0.82-1.69)	1.43 (1.13-1.82)	1.01 (0.91-1.12)	1.02 (0.93-1.12)	<.001
P value	.37	.003	.85	.63	

Donor Group	HR per Unit Transfused (95% CI) <sup>b</sup>
<b>KPNC (n = 34 662)</b>	
Female	0.99 (0.96-1.03)
Previously pregnant <sup>d</sup>	1.00 (1.00-1.01)
Sex-discordant	1.02 (0.99-1.05)
<b>REDS-III (n = 93 724)</b>	
Female	1.00 (0.99-1.01)
Previously pregnant <sup>d</sup>	1.01 (0.98-1.03)
Sex-discordant	0.99 (0.98-1.00)
<b>SCANDAT (n = 918 996)</b>	
Female	1.00 (0.99-1.00)
Parous	1.00 (1.00-1.01)
Sex-discordant	1.00 (0.99-1.00)

*Caram-Deelder et al, JAMA, 2017*

*Edgren et al, JAMA, 2019*

# Changes in donor parity over time

**Table 4. Results From Analysis Using the SCANDAT Database, Comparing Survival of Patients Transfused Before and After Female Donors Deliver Their First Child**

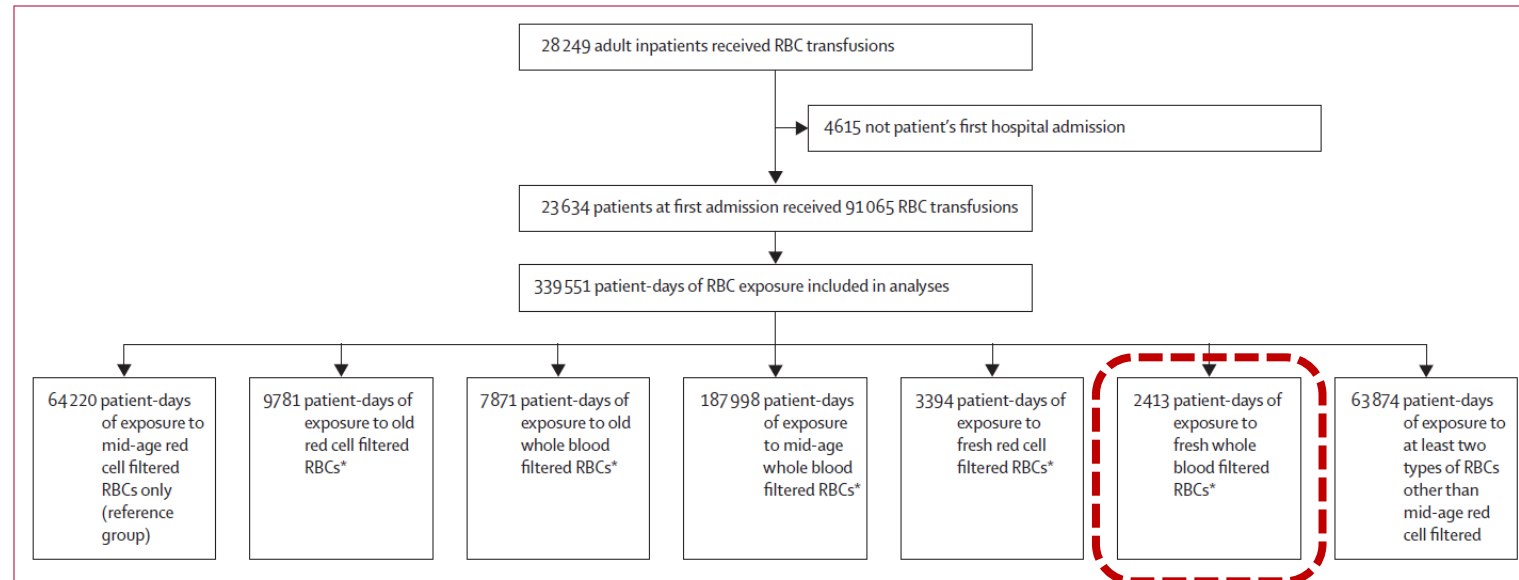
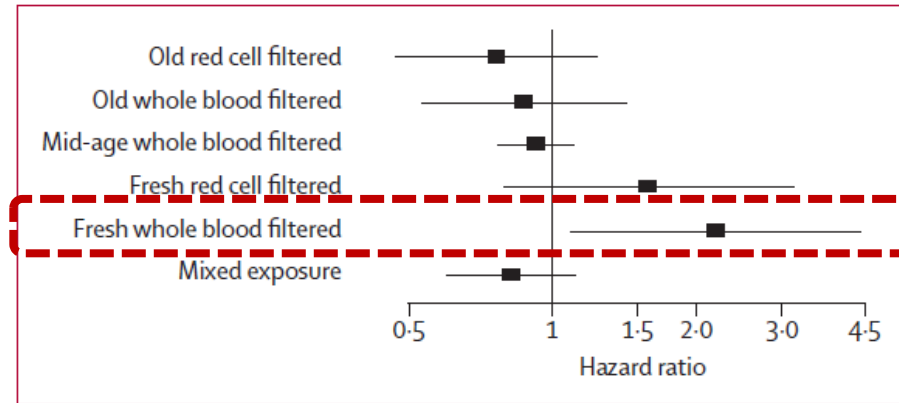
	Donor Parity			
	Nulliparous	1 Delivery	2 Deliveries	≥3 Deliveries
Donor recipients <sup>a</sup>	78 594	39 383	19 275	4092
Person-years	54 542	26 437	12 558	2577
Deaths	32 219	16 278	7801	1640
HR (95% CI) <sup>b</sup>	1 [Reference]	1.01 (0.99-1.03)	1.01 (0.97-1.04)	1.01 (0.94-1.08)

Abbreviations: HR, hazard ratio; SCANDAT, Scandinavian Donations and Transfusions.

<sup>a</sup> A total of 110 996 unique patients were included in this analysis, but because some patients received blood from more than 1 female donor who changed parity status, the sum is higher at 141 344.

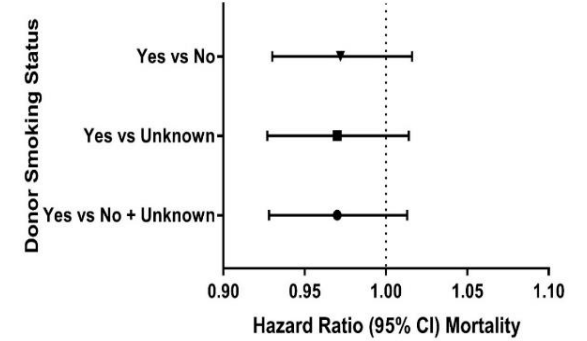
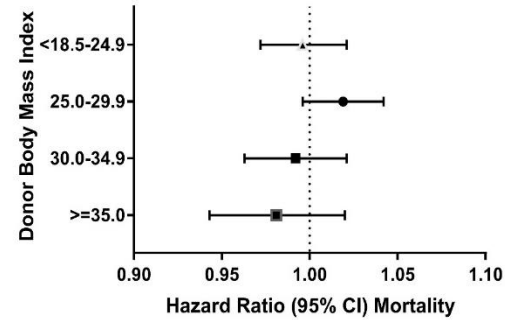
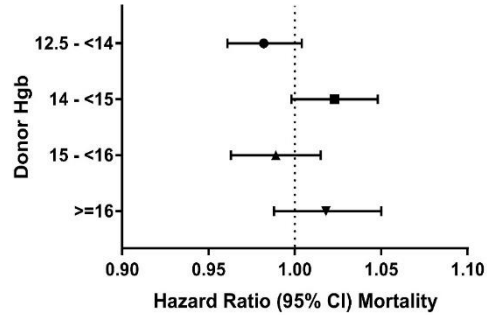
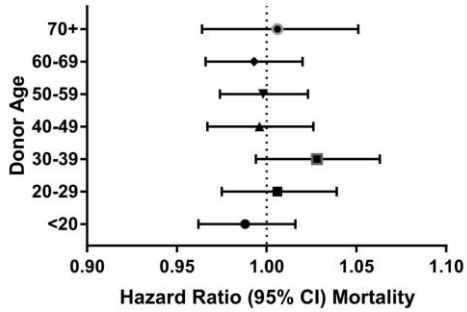
<sup>b</sup> HRs were calculated using a 2-step process, first adjusting for calendar year, hospital, patient age, patient sex, as well as patient Charlson comorbidity index and then considering the association of donor parity using a stratified Cox model, with the aforementioned adjustment included as an offset.

# Component modifications & mortality?

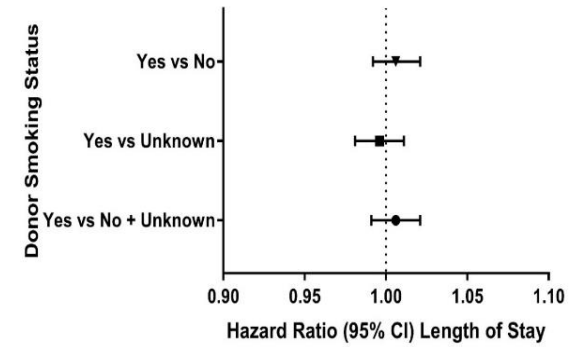
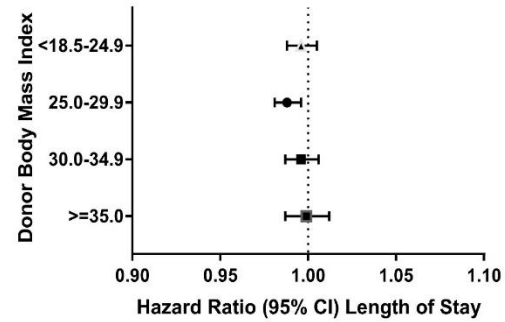
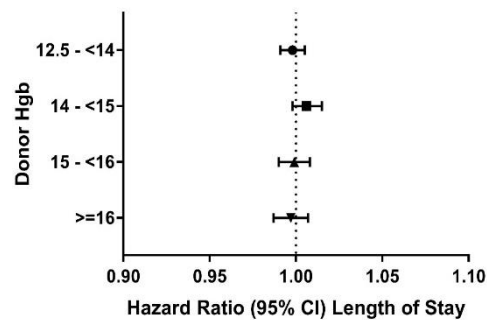
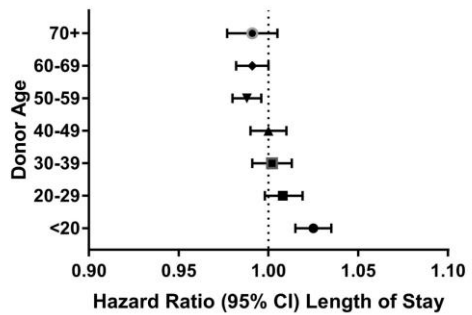


# Other blood donor factors or outcomes?

## Mortality



## Length of Stay



**Donor age**

**Donor hemoglobin**

**Donor body mass index**

**Donor smoking**

Open access

Protocol

BMJ Open

## ITADS: AN INNOVATIVE TRIAL ASSESSING DONOR SEX ON RECIPIENT MORTALITY

SPONSOR

Ottawa Hospital Research Institute  
(Other)

OVERALL STATUS

Active, not  
recruiting

CT.GOV ID

NCT03344887

COLLABORATOR

Canadian Institutes of Health Research (CIHR) (Other), Canadian Blood Services (Other)

8,850

ENROLLMENT

1

LOCATION

2

ARMS

42

ANTICIPATED  
DURATION (MONTHS)

210.9

PATIENTS PER SITE PER  
MONTH

Randomised  
controlled trial of donor sex on recipient mortality in  
transfusion-dependent thalassaemia: the  
ITADS trial

David P Acker,<sup>5</sup>  
Shinya Ohata,<sup>9</sup>  
David G. H. Mann,<sup>1</sup>

Retrospective cohort study using EHR data on 23,194 transfusion recipients who received one or more single-unit RBC transfusions from 2008-2016 at a Kaiser Permanente Northern California facility

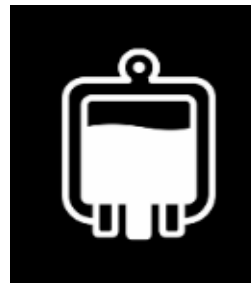
## Factors affecting hemoglobin increments in transfusion recipients

### Blood Donor



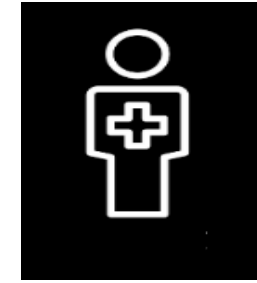
- Male blood donor
- Donor age < 70
- (+) Rh-D status

### Blood Component



- Apheresis blood collection
- Additive solution
- Gamma irradiation
- Storage duration > 35 days

### Transfusion Recipient



- Female recipient
- Recipient age
- (+) Rh-D status
- Lower body mass index
- Lower hemoglobin level

# Gamma Irradiation and Storage Duration

<b>Un-irradiated</b>				
<b>Storage Duration (days)</b>	1-21 (n=9,580)	22-28 (n=11,791)	29-35 (n=7,738)	36-42 (n=6,433)
<b>Pre-TX Hb</b>	8.10 (0.88)	8.04 (0.87)	8.04 (0.85)	7.92 (0.87)
<b>Post-TX Hb</b>	9.14 (1.17)	9.08 (1.15)	9.09 (1.12)	8.97(1.12)
<b>Post-TX Inc</b>	<b>1.05 (0.90)</b>	<b>1.05 (0.89)</b>	<b>1.07 (0.87)</b>	<b>1.07 (0.85)</b>
<b>Irradiated</b>				
<b>Storage Duration (days)</b>	1-21 (n=828)	22-28 (n=643)	29-35 (n=539)	36-42 (n=467)
<b>Pre-TX Hb</b>	7.65 (0.82)	7.77 (0.78)	7.78 (0.81)	7.78 (0.80)
<b>Post-TX Hb</b>	8.59 (1.12)	8.76 (1.19)	8.68 (1.06)	8.69 (1.07)
<b>Post-TX Inc</b>	<b>0.96 (0.82)</b>	<b>1.03 (0.96)</b>	<b>0.96 (0.73)</b>	<b>0.98 (0.79)</b>



# KPNC - Donor-component-recipient linkage

Hemoglobin increments for donor & recipient sex and gamma irradiation – mean in g/dL (SD)				
	Male Blood Donor		Female Blood Donor	
	Unirradiated	Irradiated	Unirradiated	Irradiated
<b>Female Recipient</b>				
Hb Increment	1.23 (0.93)	1.18 (0.96)	1.14 (0.89)	1.08 (0.84)
<b>Male Recipient</b>				
Hb Increment	0.93 (0.83)	0.88 (0.79)	0.88 (0.84)	0.74 (0.77)
Hemoglobin increments for donor & recipient sex and collection method – mean in g/dL (SD)				
	Whole blood collection		Apheresis collection	
	Male Donor	Female Donor	Male Donor	Female Donor
<b>Female Recipient</b>				
Hb Increment	1.29 (0.93)	1.14 (0.89)	1.10 (0.90)	1.04 (0.82)
<b>Male Recipient</b>				
Hb Increment	0.97 (0.82)	0.88 (0.83)	0.85 (0.82)	0.79 (0.86)

# Modeling hemoglobin increments

## Hb increment (g/dL) after transfusion for Hb level of 7 g/dL

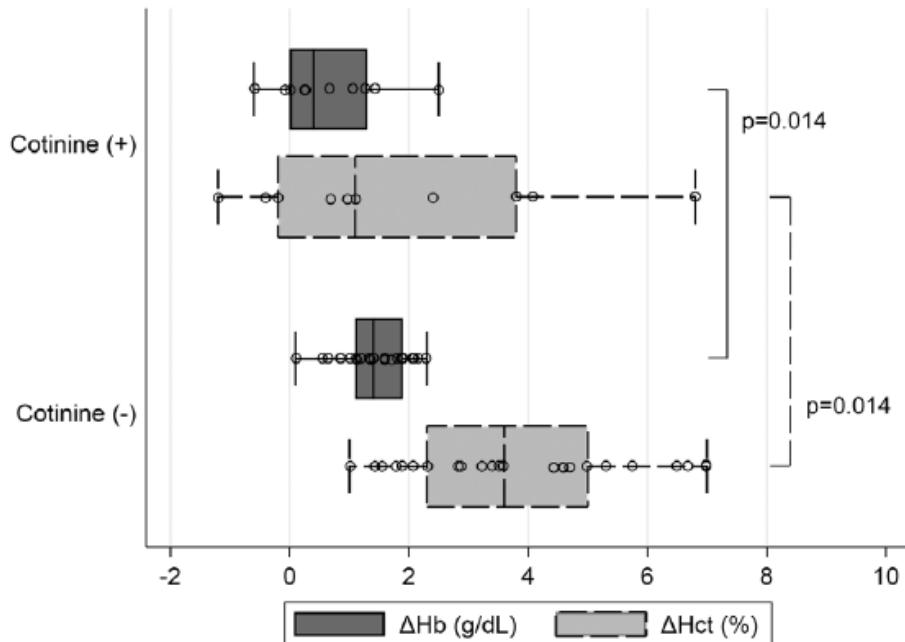
- Female donor (0)
- Don/Rec Rh-D neg (-0.06)
- Apheresis collection (0)
- Irradiated unit (-0.08)
- Additive solution 3 (-0.06)
- 60-yo old male recip. (0)
- BMI – 30 (-0.5)

**0.59 g/dL Hb increment**

- Male donor (+0.1)
- Don/Rec Rh-D pos (0)
- Whole blood coll. (+0.16)
- Unirradiated unit (0)
- Additive solution 1 (0)
- 85-yo old female (+0.4)
- BMI – 18 (-0.3)

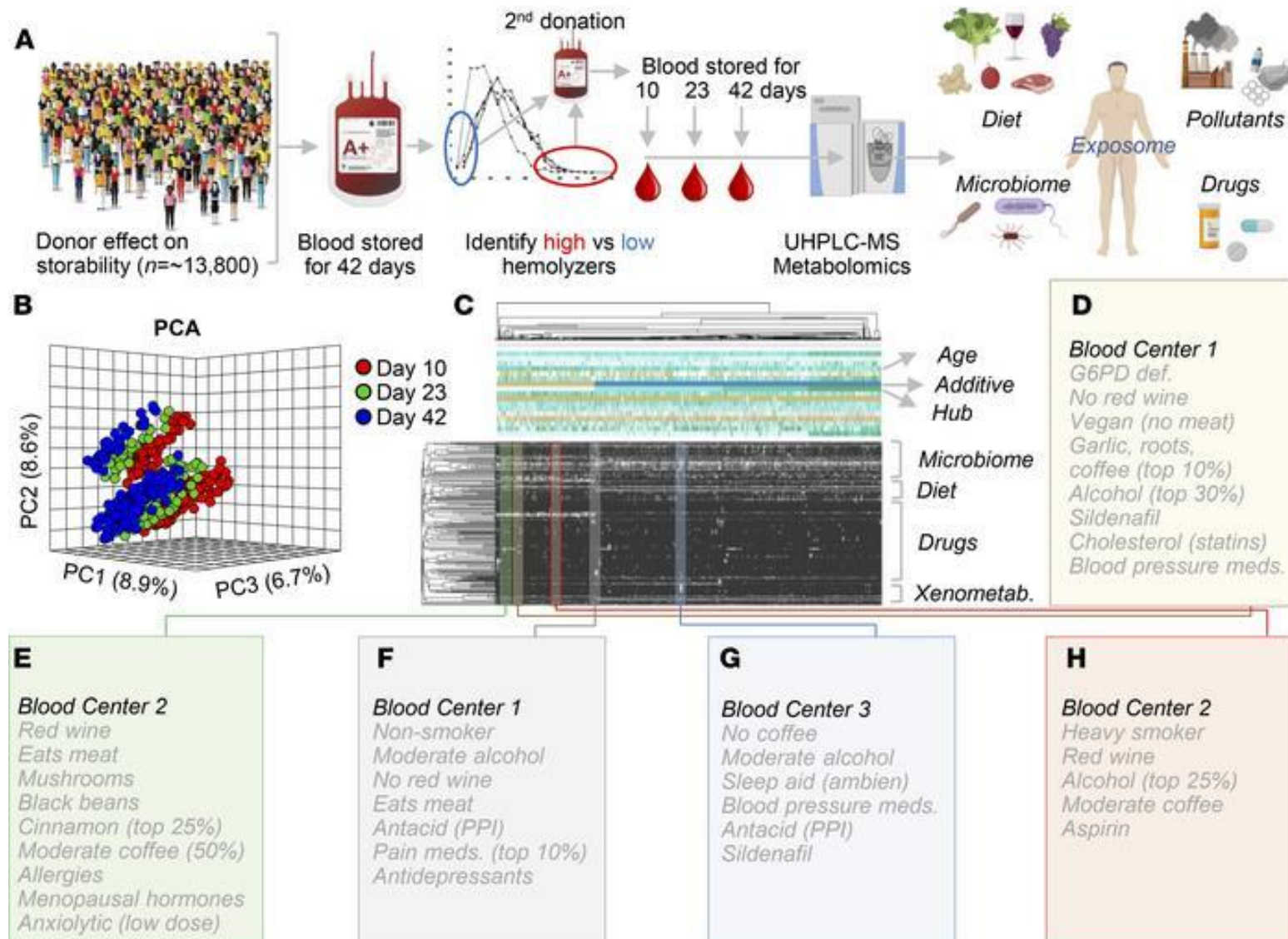
**1.65 g/dL Hb increment**

# Donor behaviors: smoking & RBC transfusion

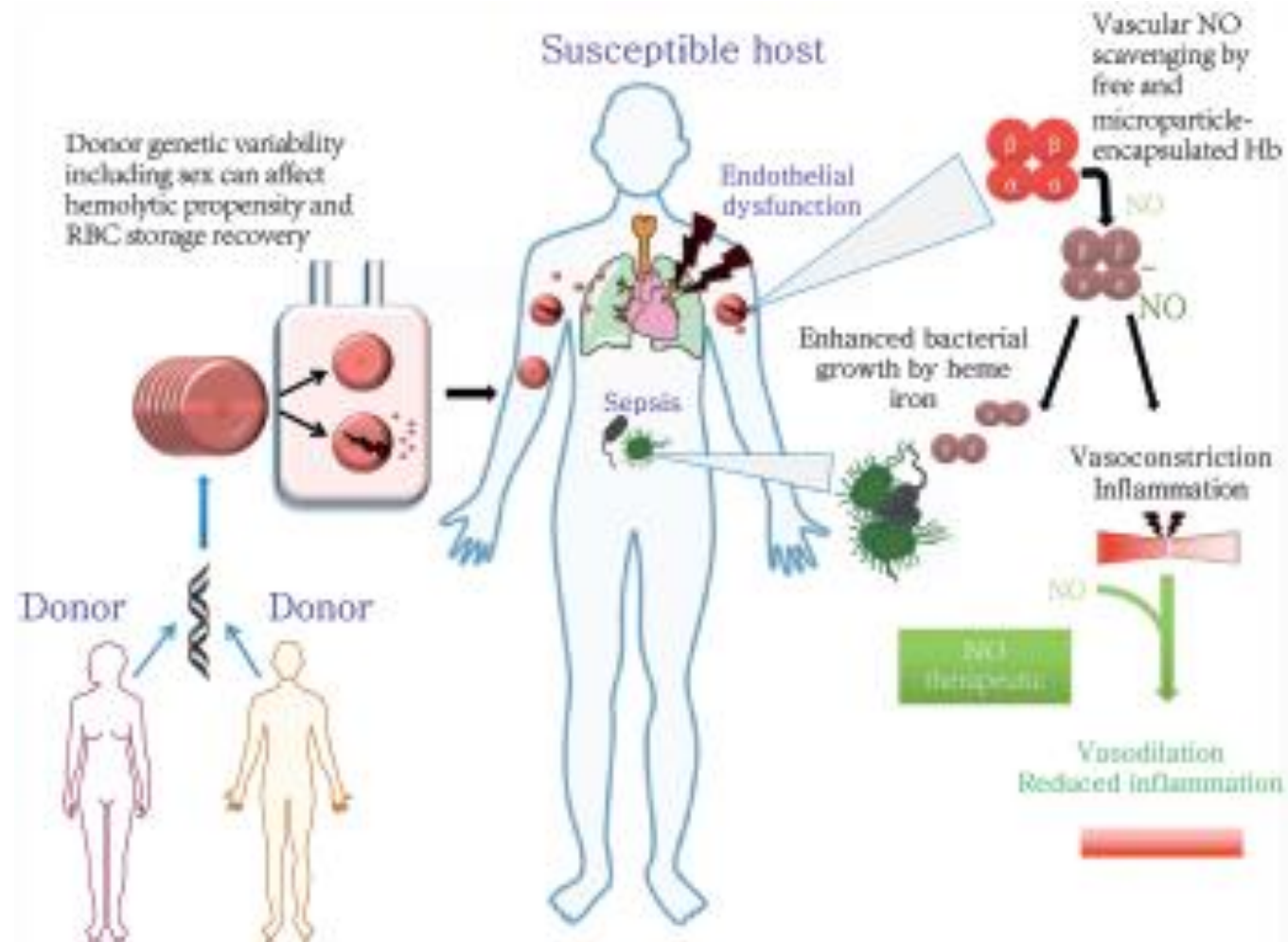


Donor & component factor	Δ Hemoglobin, g/dL (SD)
Non-smoker, unirradiated	1.03 (0.92)
Smoker, unirradiated	1.05 (0.98)
Non-smoker, irradiated	0.94 (0.83)
Smoker, irradiated	0.74 (0.80)

# Blood donor "Exposome"



# Donor genetic variations can affect the hemolytic propensity and recovery of RBC

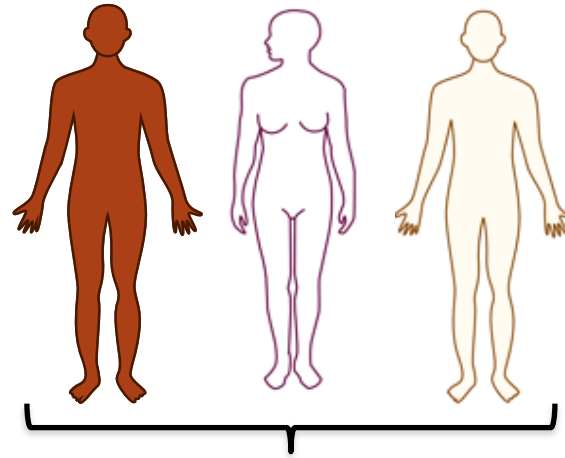






# Associations between RBC-omics donors & hemolysis with storage

**Blood donors  
(n=13,403)**



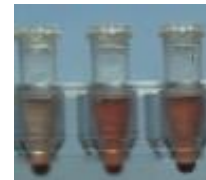
**Red blood cell storage (39-42 days)**



**Storage hemolysis**



**Osmotic hemolysis**



**Oxidative hemolysis**



**Lower  
hemolysis**



- Sex (female)

- Ethnicity (African American)
- Sex (female)

- Sex (female)
- Donor age
- Prior donation

**Higher  
hemolysis**

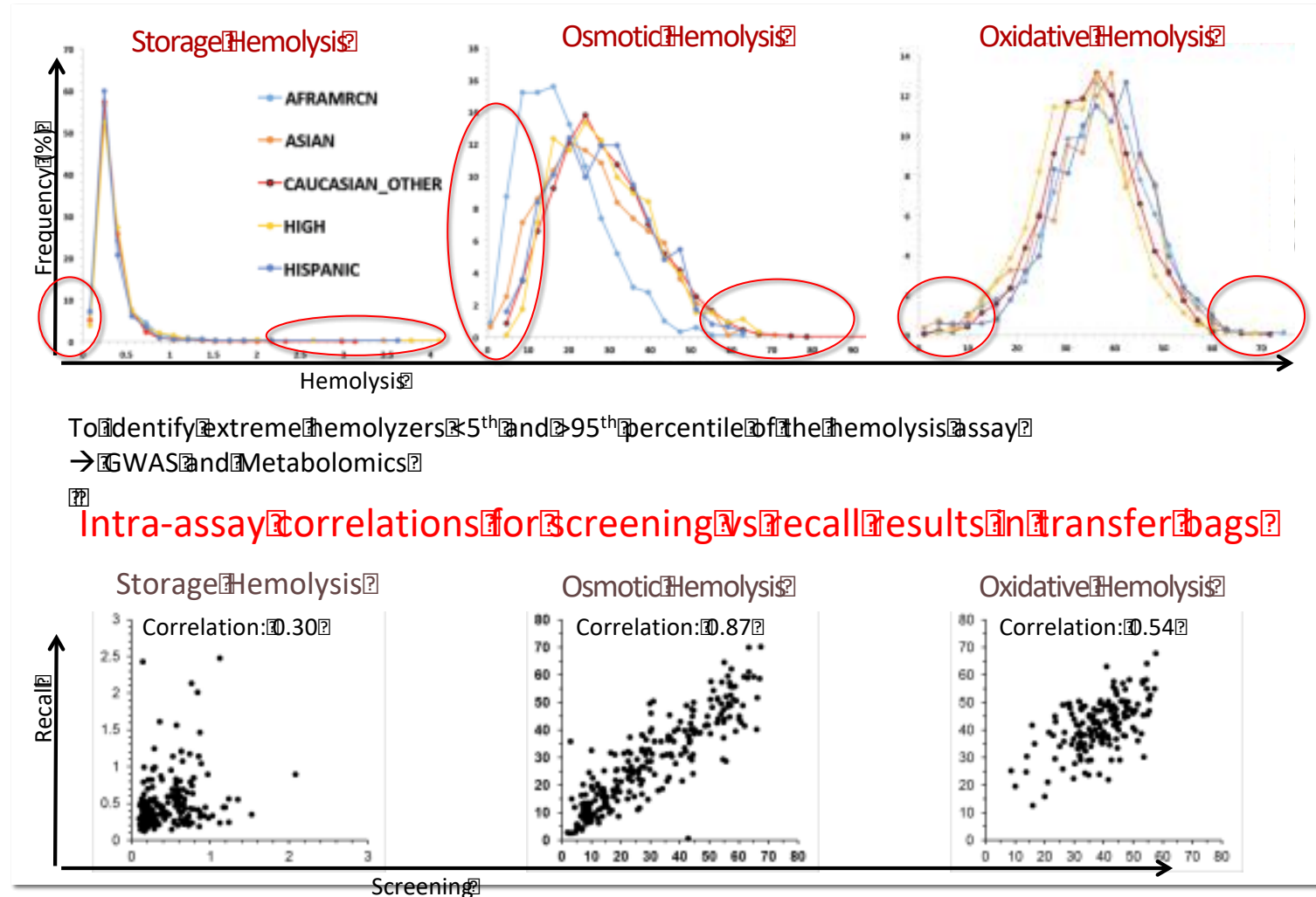


- Sex (male)
- Ethnicity (Asian/African American)

- Sex (male)
- Donor age

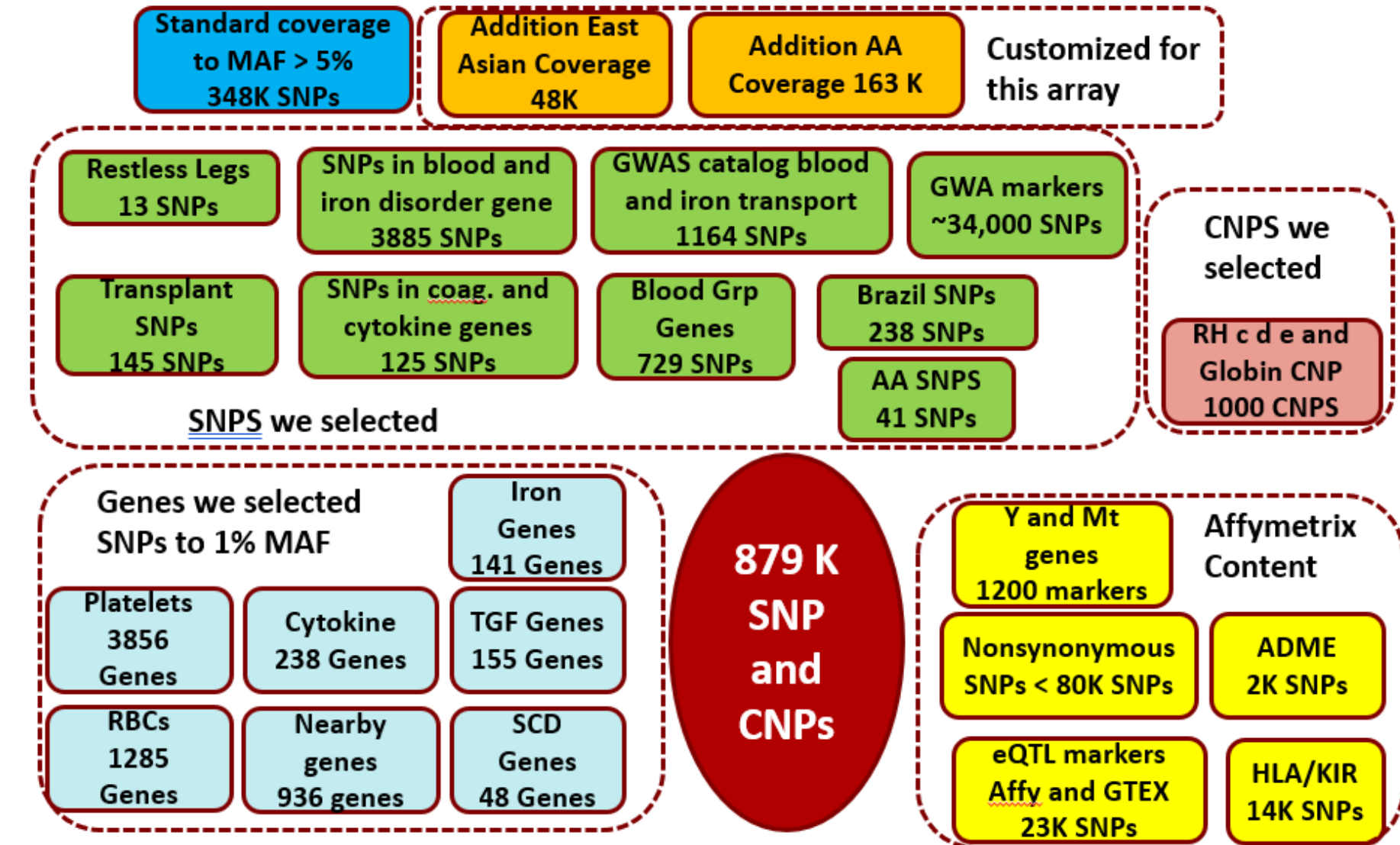
- Sex (male)

# Extreme hemolyzers during screening – Reproducibility at recall





# REDS-III Transfusion Medicine Array

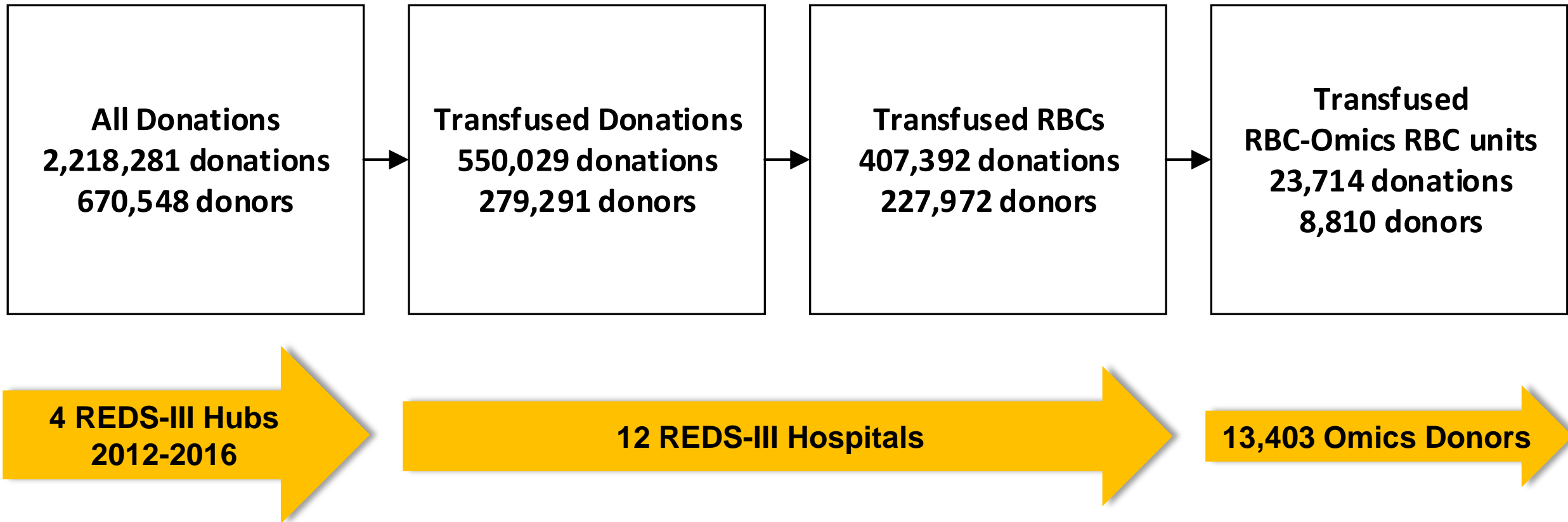


Candidate genes or SNPs with function or disease association, if known, showing 15 genome wide significant hits ( $p < 5 \times 10^{-8}$ )\* for osmotic hemolysis and 4 genome wide significant hits\*\* for oxidative hemolysis from RBC-Omics donors.

Examples of Candidate Genes or SNPs	Function / Disease Association	Type of Hemolysis
ANK1 (Ankyrin 1)	Hereditary Spherocytosis	Osmotic*
AQP1 (Aquaporin 1)	Water channel protein	Osmotic*
SPTA1 (Spectrin Alpha)	Hereditary Spherocytosis/Elliptocytosis	Osmotic*
PIEZ01	Mechanosensitive ion channel component 2	Osmotic*
HK1 (Hexokinase)	Mitochondrial membrane protein / hemolytic anemia	Osmotic*
SWAP70/LOC440028	SWAP switching B-cell complex	Osmotic*
MYO9B (Myosin IXB)	Myosin	Osmotic*
IKZF2/DDC/GRB10	Zink finger/Centromeric heterochromatin/ cell surface receptor kinases	Osmotic*
MIR4289	MircroRNA of unknown significance	Osmotic*
CNTN5/ARHGAP42	Contactin 5/ Rho GTPase activating protein 42	Osmotic*
SH2B3/BRAP/MAPKAPk5/NAA25/Others	SH2B adaptor protein 3/ BRCA1 associated protein	Osmotic*
HBA2	Hemoglobin subunit alpha 2	Osmotic*
SLC4A1/UBTF	Solute carrier family 4 (anion exchanger)/ Transcription factor, RNA polymerase I	Osmotic*
ANTXRLP1	Pseudogene	Osmotic*
EPB41 (Erythrocyte Membrane Protein Band 4.1)	Elliptocytosis-1	Osmotic*
ESYT2	Extended synaptotagmin-like protein 2	Osmotic
G6PD A-	G6PD deficiency	Oxidative**/Osmotic
GPX4 (Glutathione Peroxidase 4)	Role in oxidative stress	Oxidative**
GLRX (Gluaredoxin)	Role in oxidative stress	Oxidative**
SEC13L4/SEC14L2/SEC14L4	SEC14 like lipid binding 4	Oxidative**
TRAK1/ULK4	Trafficking protein, kinesin binding 1/ kinase 4	Oxidative



# REDS-III donor & donation linkages



# Prevalence of selected SNPs associated with osmotic hemolysis in donors and transfused RBC recipients

	<b>AQP1</b>	<b>IKZF1</b>	<b>ANK1</b>	<b>HK1</b>	<b>GPX4</b>	<b>GLRX</b>
<b>VRI RBC-Omics donors (n=3,129)</b>						
Homozygous dominant	75.2%	28.8%	48.2%	86.6%	36.1%	91.4%
Heterozygous recessive	22.4%	48.4%	40.1%	12.8%	47.3%	8.3%
Homozygous recessive	2.4%	22.8%	11.7%	0.6%	16.5%	0.3%
<b>KPNC RBC transfusions (n=3,434)</b>						
Homozygous dominant	71.2%	27.9%	51.3%	84.3%	34.9%	89.3%
Heterozygous recessive	25.7%	49.8%	39.6%	15.0%	46.8%	10.5%
Homozygous recessive	3.1%	22.3%	9.2%	0.8%	18.3%	0.3%

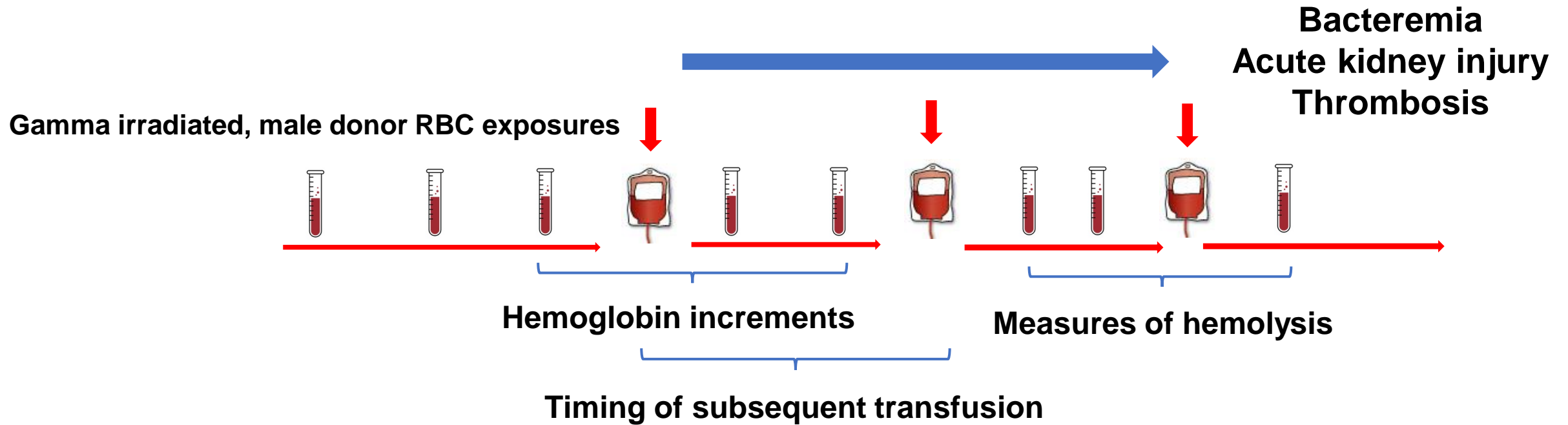
# Need for additional RBC transfusion-1 week

<b>Decreased Hb dose</b>	<b>Increased Hb dose</b>
<ul style="list-style-type: none"><li>• Female donor</li><li>• Donor age &gt; 70</li><li>• Apheresis collection</li><li>• Irradiated unit</li></ul> <p><b><u>47% transfused - 1 week</u></b></p>	<ul style="list-style-type: none"><li>• Male donor</li><li>• Donor age &lt; 70</li><li>• Whole blood collection</li><li>• Unirradiated unit</li></ul> <p><b><u>28% transfused - 1 week</u></b></p>

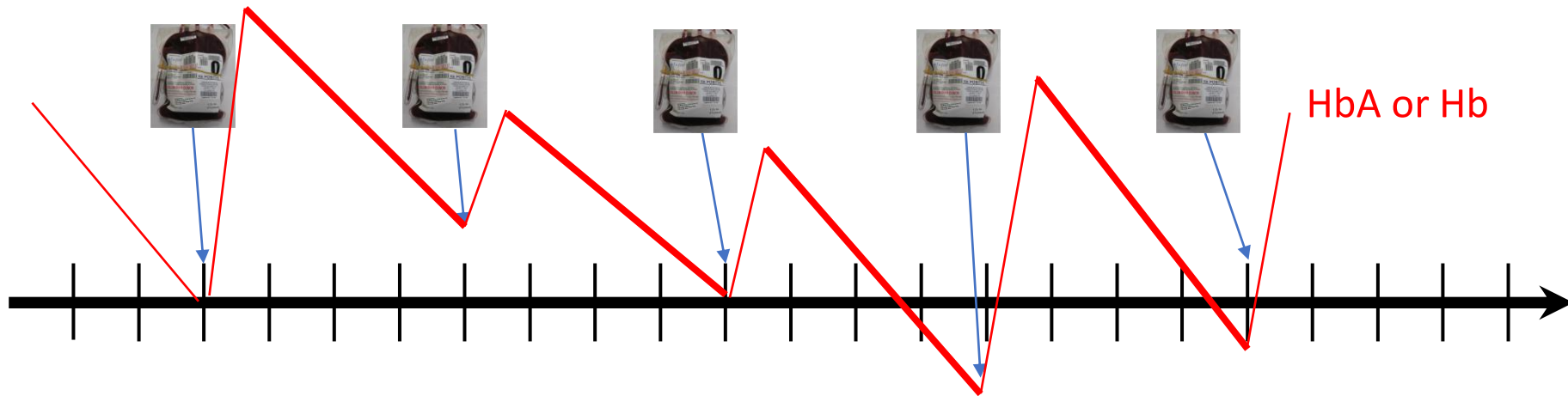


**19% absolute difference**  
**40% relative rate difference**

# Longitudinal outcomes of RBC transfusion



# REDS-IV-P: Red Blood Cell – IMProving trAnsusions for Chronically Transfused recipients (RBC – IMPACT)



Specific genetic donor factors influence RBC survival of units transfused to patients with SCD (measured by HbA) and Thalassemia (measured by total Hb)



# REDS-III Linked donor-component-recipient database (2013-2016)

- 4 blood centers and 12 hospitals
- Donors and donations
  - Over 2 M successful donations from ~ 650,000 donors
    - Demographics, prior donation history
- Components and modifications
  - Over 5.5 M components
    - Collection method, processing and modifications: e.g., leukoreduction, additive solutions, gamma irradiation, volume reduction, storage age
- Recipients (inpatient and outpatients)
  - Over 1.5 M encounters from ~ 750,000 unique patients

[www.biolincc.nhlbi.nih.gov](http://www.biolincc.nhlbi.nih.gov)

# Summary

- Linked blood donor-component-recipient databases provide unique opportunities to study transfusion effectiveness
- Donor genotype and phenotype data will allow us to better understand mechanisms of clinical associations
- Studying granular longitudinal outcomes of transfusion are relevant to understand the role of donor and component covariates
- Ongoing collaboration and corroboration are critical

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