Glycans and Blood Homeostasis

Glycobiology/Glycochemistry course, VCU

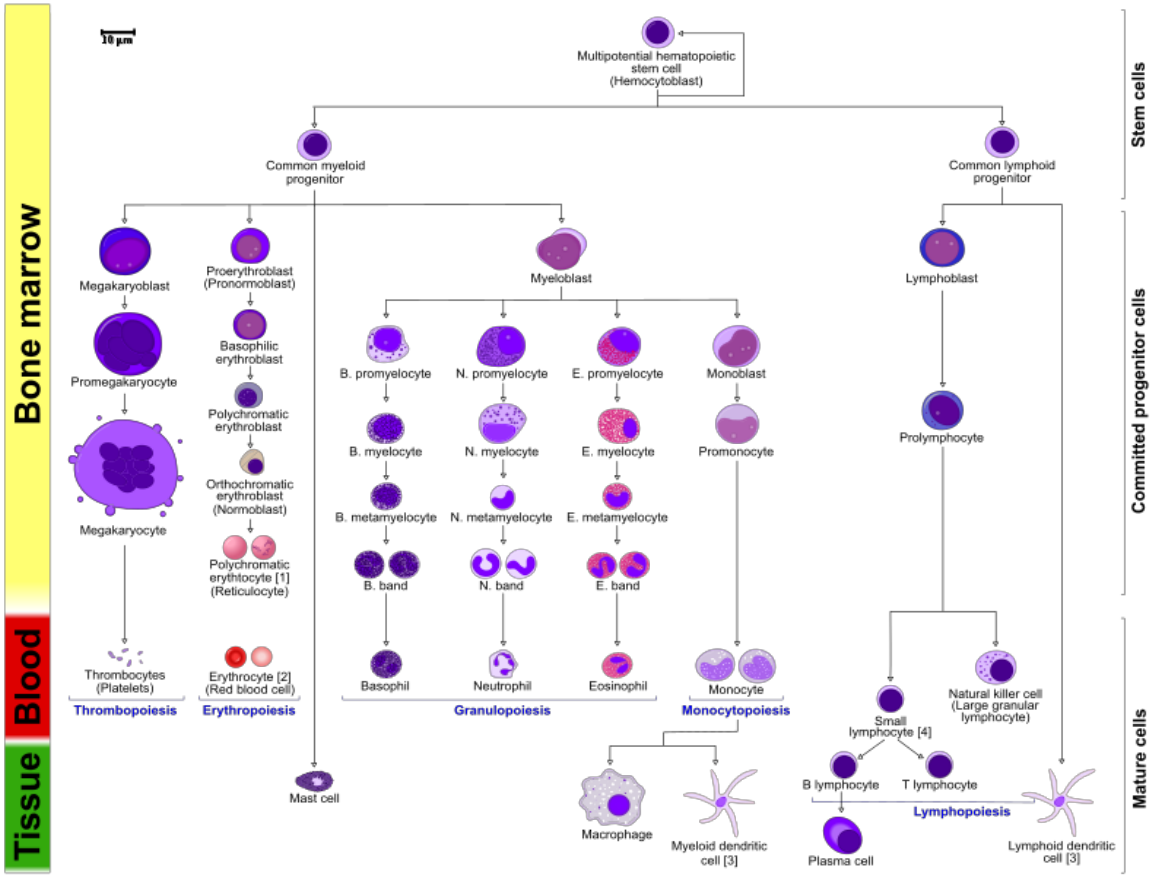
K12 Scholar Educational Course

Karin Hoffmeister, MD

March 4th 2019

Hematopoiesis

- Is the formation of blood cellular components
- Takes place in the bone marrow
- Cellular blood components are derived from hematopoietic stem cells
- In a healthy adult person, approximately 10^{11}–10^{12} new blood cells are produced daily to maintain steady state levels in blood

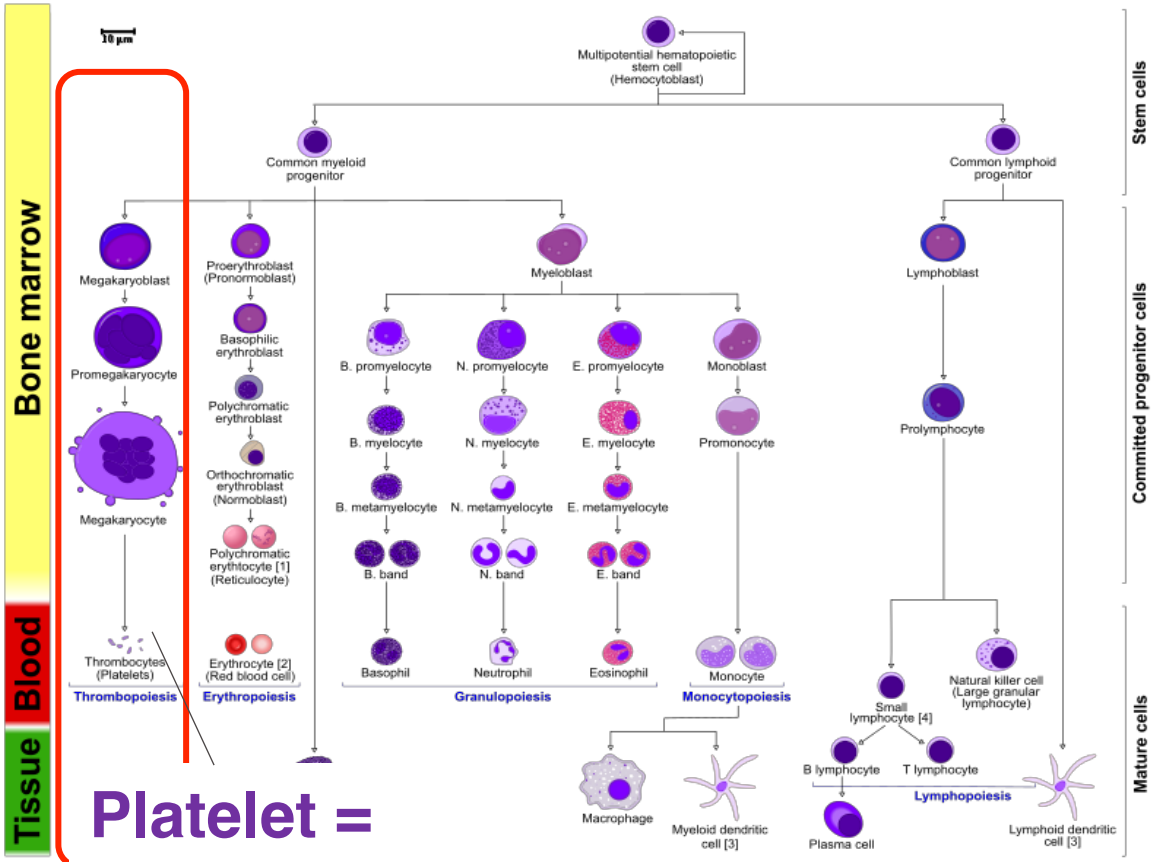


Wikipedia

Hematopoiesis

Blood cells are divided into three lineages:

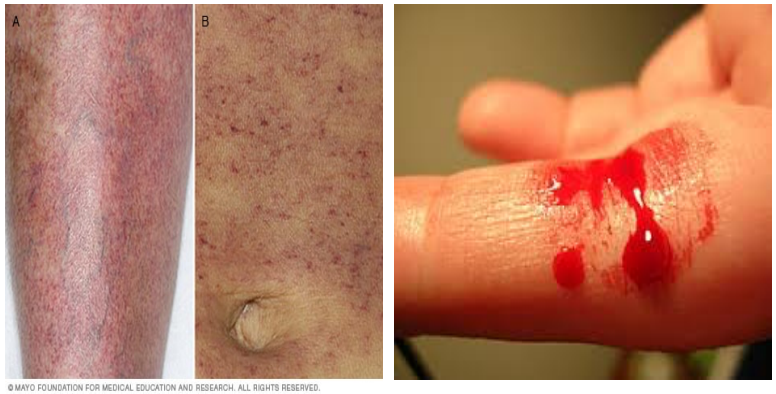
- ❑ Erythropoiesis: Red blood cells
- ❑ Lymphopoiesis: The lymphoid lineage is composed of T-cells, B-cells and natural killer cells.
- ❑ Myelopoiesis: Granulocytes, megakaryocytes and macrophages
- ❑ Focus: Megakaryocytes and Platelets



Platelet = Thrombocyte

Wikipedia

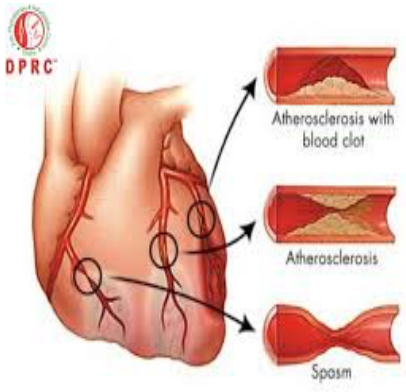
Why focus on megakaryocytes and platelets?



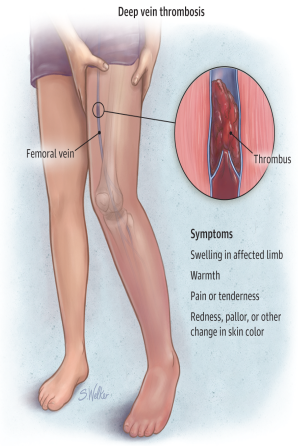
Thrombocytopenia: Petechiae

Injury induced bleeding

- ✓ **Platelets are anucleated;**
- ✓ **Platelets circulate for ~10 days;**
- ✓ **Normal platelet count: 150K-400K per microliter;**
- ✓ **Increase (thrombocytosis) or decrease (thrombocytopenia) in platelet numbers can be associated with thrombosis or bleeding, respectively.**

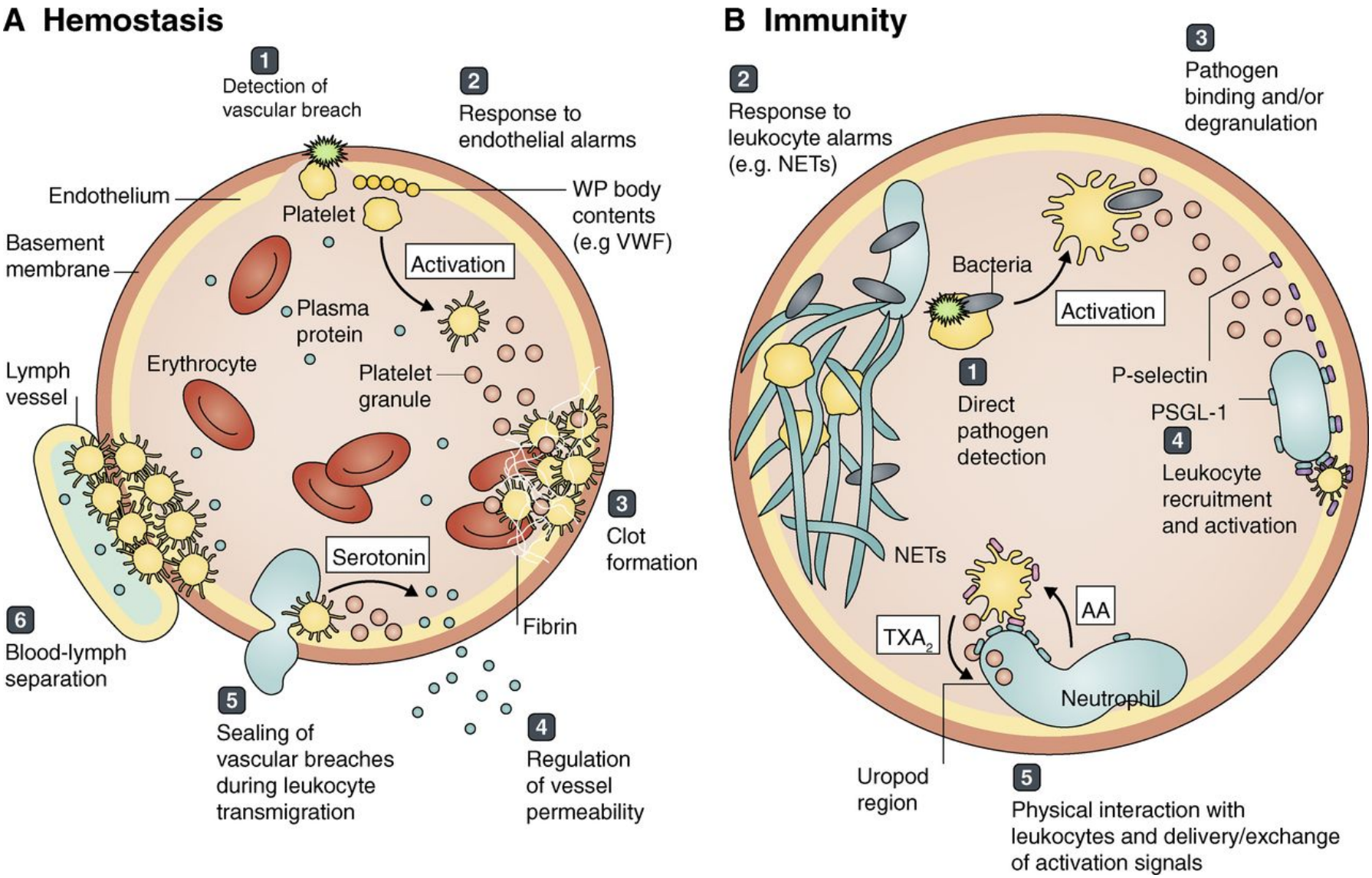


Arterial thrombosis: Myocardial Infarct



Deep Venous Thrombosis

Major platelet tasks in hemostasis and immunity.

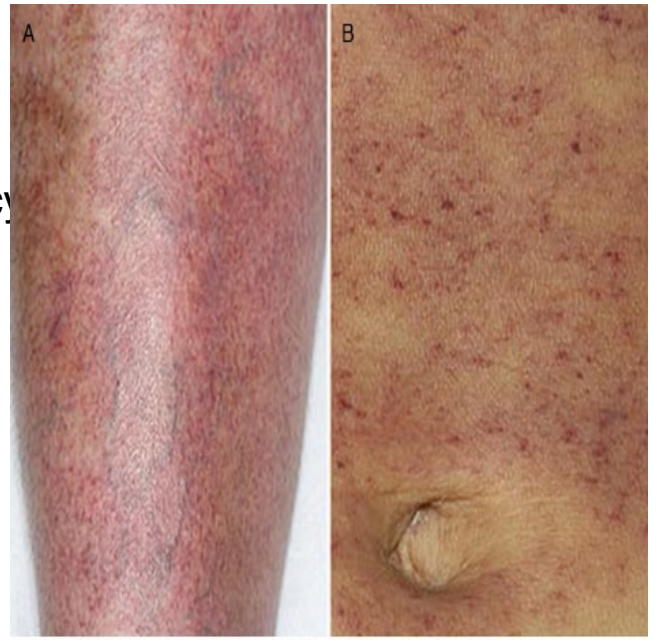


Jackson LiangYao Li et al. J Exp Med 2017;214:2193-2204

Causes of Thrombocytopenia

- **Bone marrow does not produce enough platelets:**
  - ✓ Leukemia
  - ✓ Lymphoma
  - ✓ Aplastic anemia
  - ✓ Vitamin deficiency anemias (vitamin B12 and folate deficiency
  - ✓ Some bone marrow disorders
  - ✓ Some chemotherapy drugs

- **Platelets become entrapped in an enlarged spleen:**
  - ✓ Liver Cirrhosis
  - ✓ Myelofibrosis
  - ✓ Gaucher disease



Complication: Bleeding

- **Immune thrombocytopenia:**
  - ✓ **Anti platelet receptor antibodies**
  - ✓ HIV infection, influenza, and other viral infections
  - ✓ Drugs such as heparin, quinine, many antibiotics (trimethoprim/sulfamethoxazole, rifampin, vancomycin)
  - ✓ ***Conditions involving disseminated intravascular coagulation (DIC) associated with complications of childbirth, cancer, sepsis due to gram-negative bacteria, traumatic brain damage***
  - ✓ Cardiopulmonary bypass surgery
  - ✓ Thrombotic thrombocytopenic purpura
  - ✓ Hemolytic-uremic syndrome
  - ✓ Paroxysmal nocturnal hemoglobinuria or paroxysmal cold hemoglobinuria

Causes of thrombocytosis and excessive thrombus formation

- ❑ **Complications:**

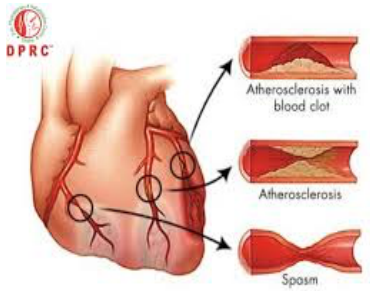
- ✓ **Venous thrombosis: DVT, renal thrombosis**

- ✓ ***Arterial thrombosis: Myocardial infarct, Stroke***

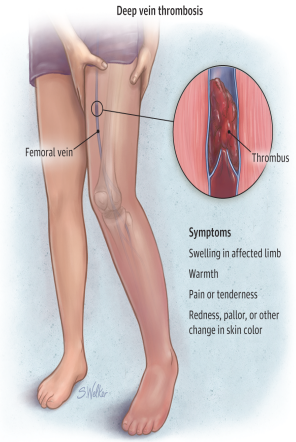
- ❑ **Causes of thrombocytosis:**

- ✓ Reactive thrombocytosis (88% to 97% in adults):
  - Acute infection
  - Chronic inflammation;
  - Tissue damage
  - Following bone marrow suppression;
  - Post surgery
  - Hemolytic anemia, thalassemia (children living in the middle east)

- ✓ Clonal thrombocythemia, i.e. myeloproliferative disease
  - Essential thrombocythemia
  - Chronic myelogenous leukemia
  - Polycythemia vera
  - Primary myelofibrosis



Arterial thrombosis: Myocardial Infarct



Deep Venous Thrombosis

Thrombocytopenia and thrombocytosis therapeutics: Therapy dependents on underlying disease

- ✓ **Platelet Transfusion**

- ✓ **Thrombopoietin mimetics**
  - TPO peptide mimetics (Romiplostim)
  - TPO nonpeptide mimetics (Eltrombopag, AKR501, LGA-4665, S-888711)
  - TPO agonist antibodies

- ✓ **Corticosteroids**

- ✓ **Heparin and other antithrombotic drugs**

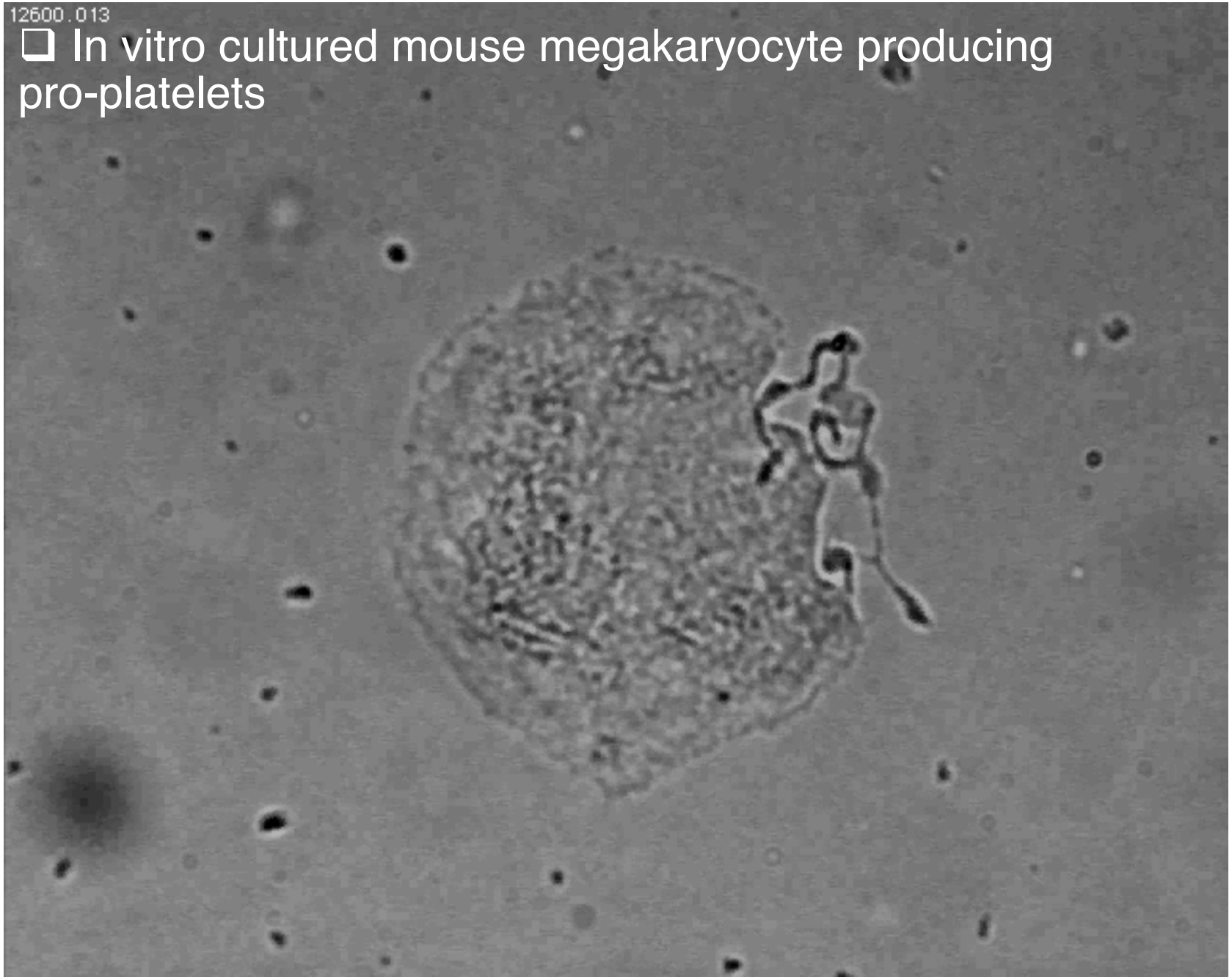
- ✓ **Chemotherapy**

- ✓ **Bone marrow transplants**

- ✓ **Others..**

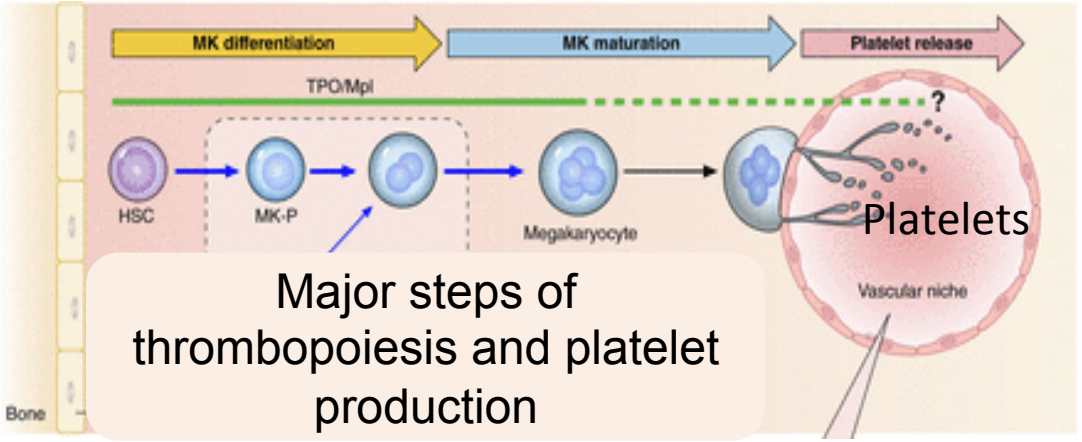
12600.013

- In vitro cultured mouse megakaryocyte producing pro-platelets



Thrombopoiesis: Process of platelet production

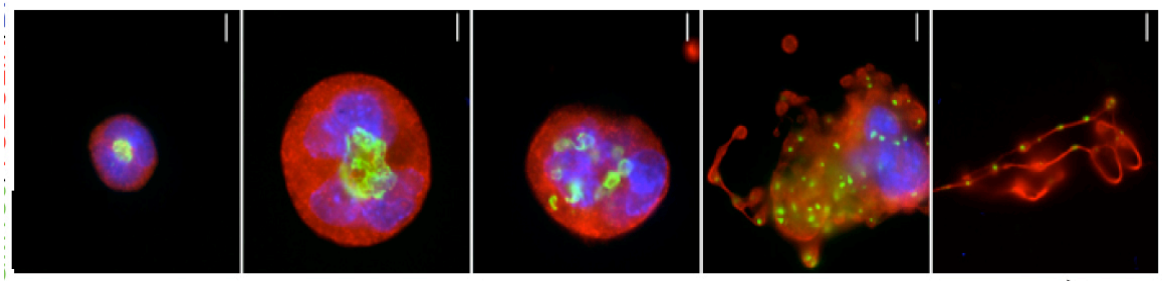
- ❑ Megakaryocytes
- ✓ Reside in the bone marrow: Largest hematopoietic cell 40-100 μM
- ✓ Undergo endomitosis with > 64 N
- ✓ Each megakaryocyte produces more than 1000 platelets
- ✓ The human body produces and removes > 10^{11} platelets per day
- ✓ Thrombopoietin (TPO) is the major hormone that induces megakaryocyte differentiation and maturation
- ✓ Last steps of thrombopoiesis require an immense membrane and organelle expansion to form and release platelets, involves ER and Golgi
- ✓ Megakaryocytes package Golgi particles into platelets
- ✓ Megakaryocytes package lysosomes and other granules into platelets



Major steps of thrombopoiesis and platelet production

Adapted from Blood 2016 127:1234-1241

DAPI Golgi Marker 130 Tubulin

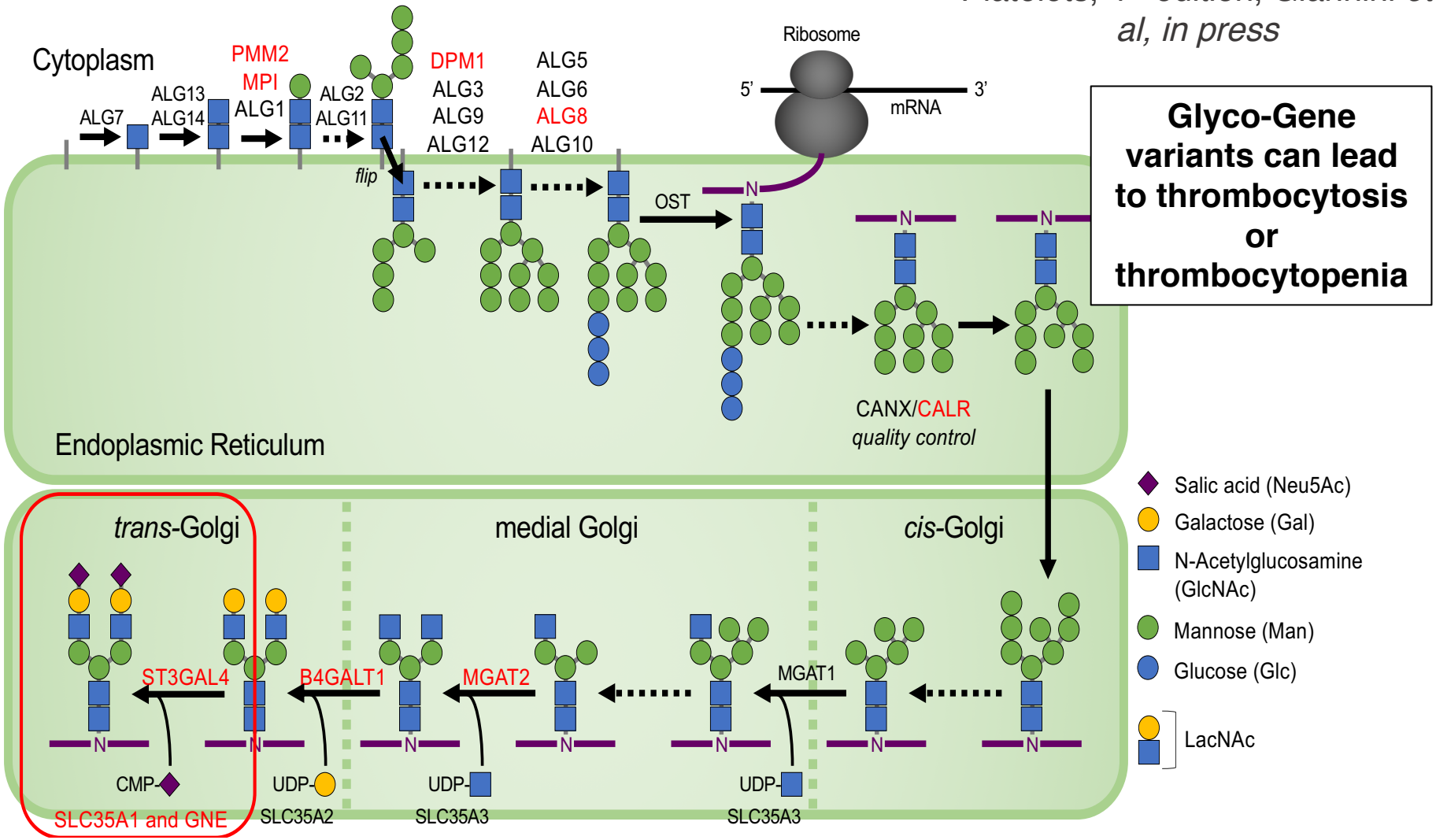


Megakaryocyte ——— Pro-platelet ⟶

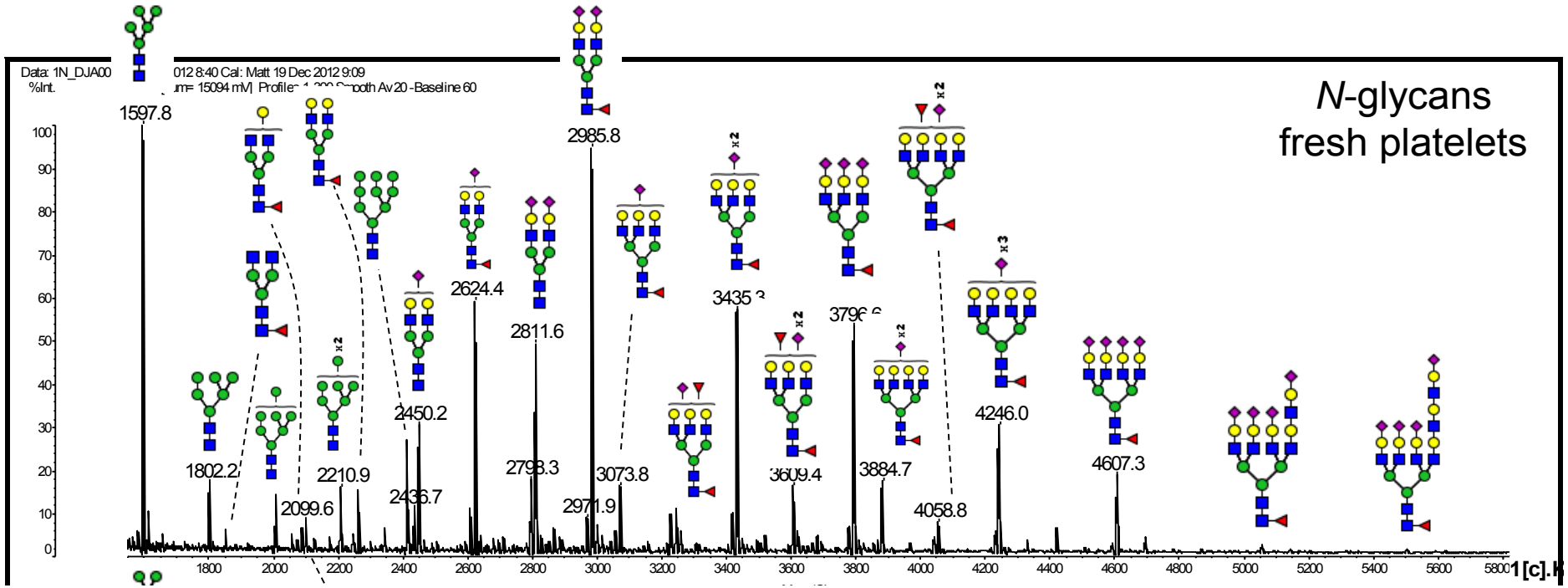
Wandall et al, Blood, 2008, Wandall et al. Blood 2012; Yadav et al, Platelets 2016

Defects in ER/Golgi enzymes and proteins affect thrombopoiesis in humans and mice.

Platelets, 4th edition, Giannini *et al, in press*



Platelets contain diverse N-linked surface glycans.



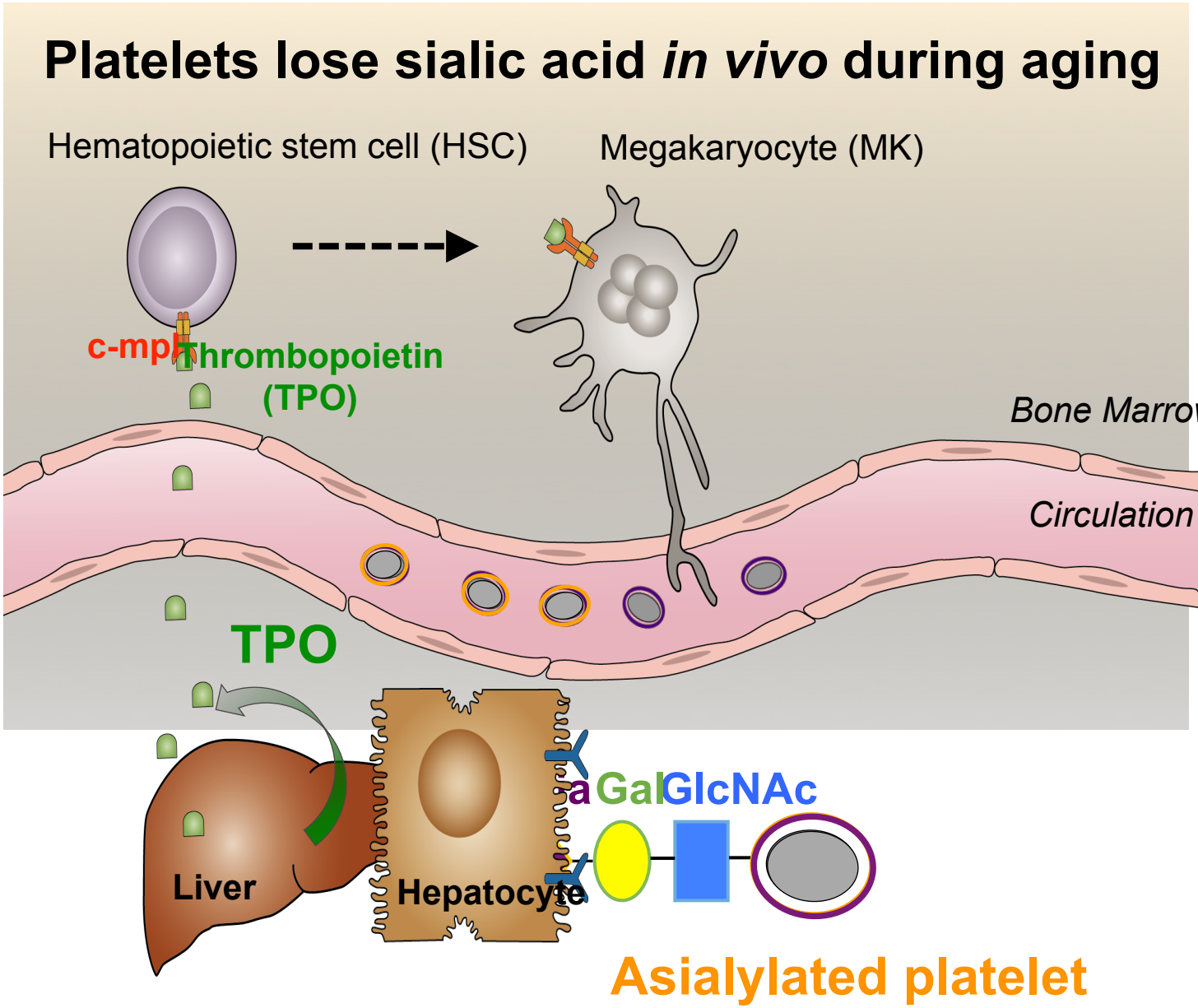
- ✓ MALDI-TOF spectrum of platelet surfaces N-linked glycan structures
- ✓ Sialic acid rich surface

Platelets, 4th edition, Giannini *et al, in press*

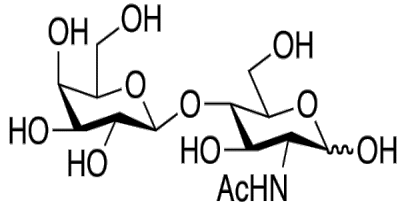
Platelet receptor copy per platelets putative glycosylation sties

Glycoprotein/Receptor	Gene	Function	Ligand	Copies	N-link. Human	N-link. Mouse	O-link. Human	O-link. Mouse
Integrins								
β3 (GPIIIa, CD61)	ITGB3			64,200	6	5	3	?
– αIIb (GPIIb, CD41)	ITGA2B	Aggregation	Fibrinogen	83,300	5	5	2	?
– αV (CD51)	ITGAV	Adhesion	Vitronectin	1,400	13	12	–	?
β1 (GPIIa, CD29)	ITGB1			10,600	14	14	4	?
– α2 (GPIa, CD49b)	ITGA2	Adhesion	Collagen	4,600	10	9	1	?
– α5 (GPIc, CD49e)	ITGA5	Adhesion	Fibronectin	1,900	15	17	2	?
– α6 (GPIc', CD49f)	ITGA6	Adhesion	Laminin	11,500	9	8	–	?
Leucine-rich glycoproteins								
GPIbα (CD42b)	GP1BA	Adhesion	VWF, αMβ2	18,900	2	–	7	?
GPIbβ (CD42c)	GP1BB			49,000	1	1	3	?
GPIX (CD42a)	GP9			32,400	1	1	1	?
GPV (CD42d)	GP5			30,200	8	7	1	?
Sialophorin (leukosialin, CD43)	SPN	?	Siglec-1	1,100	1	1	25	?
Immunoreceptors								
GPVI	GP6	Activation	Collagen	9,600	1	2	2	?
CLEC-2	CLEC1B	Activation	Podoplanin	3,700	3	4	–	–
FcγRIIA (CD32A)	FCGR2A	Activation	IgG (Fc)	1,000	2	N/A	–	N/A
PECAM-1 (CD31)	PECAM1	Inhibition	PECAM-1	9,400	9	7	–	–
G protein-coupled receptors								
PAR1	F2R	Activation	Thrombin	?	5	N/A	–	N/A
PAR4	F2RL3	Activation	Thrombin	1,100	1	1	–	–
P2X1	P2RX1	Activation	ADP	1,400	4	4	–	–
P2Y1	P2RY1	Activation	ADP	?	4	4	–	–
P2Y12	P2RY12	Activation	ADP	?	2	2	–	–
TP	TBXA2R	Activation	TXA2	?	2	2	–	–
Tetraspanins								
CD9	CD9	?	?	8,000	2	1	–	–
CD63	CD63	?	?	2,200	3	4	–	–
Others								
GPIV (CD36)	CD36	Activation	LDL (oxidized)	16,700	10	8	1	?
P-selectin (CD62P)	SELP	Adhesion	PSGL-1	8,900	12	5	4	?
Mpl (CD110)	MPL	Activation	Thrombopoietin	1,600	4	1	1	?

Platelets lose sialic acid *in vivo* during aging



- Platelets loose sialic acid during their lifespan

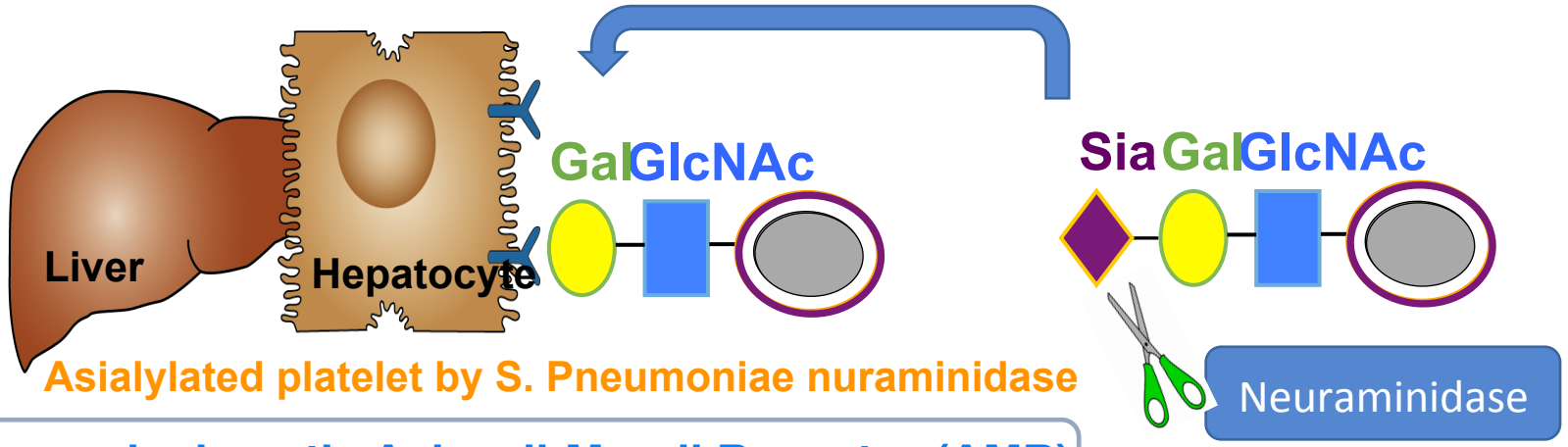


Platelet N-Acetyl-Lactosamine ligand for the Ashwell Morell Receptor

Clearance by hepatic Ashwell-Morell Receptor (AMR)

Jansen *et al.*, Blood 2012; Rumjantseva *et al.*, Nat Med 2009; Sørensen *et al.*, Blood 2009; Grozovsky *et al.*, Nat Med 2015

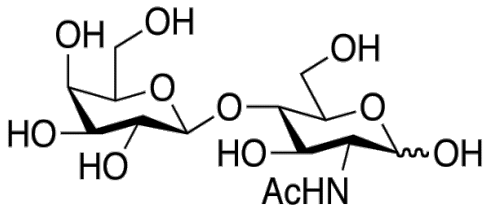
AMR clearance of activated platelets attenuates the coagulopathy associated with sepsis caused by S. Pneumoniae



Asialylated platelet by S. Pneumoniae nuraminidase

Clearance by hepatic Ashwell-Morell Receptor (AMR)

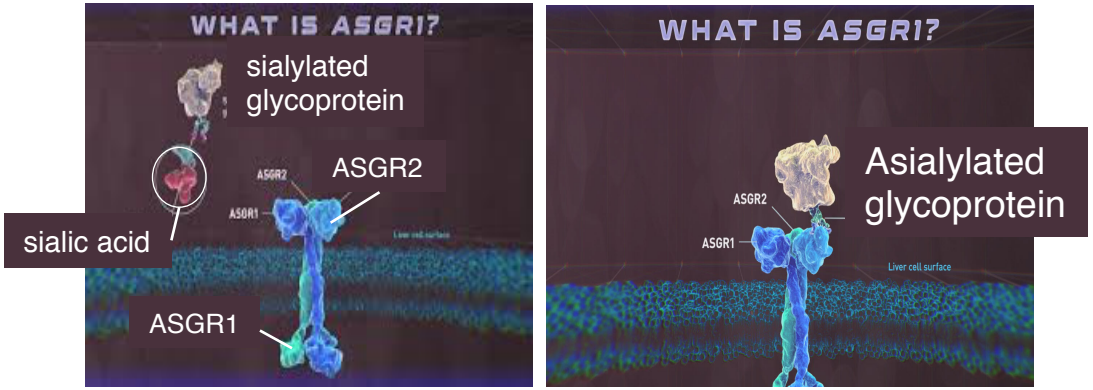
- ✓ Streptococcus Pneumoniae neuraminidase cleaves platelets sialic acid;
- ✓ Asialylation of platelets leas to formation of pathologic thrombus formation in microvasculature (DIC) and associated complications
- ✓ Removal of asialylated platelets circulation during S. Pneumoniae infection in mice protects from DIC and reduces lethality in mice



Platelet N-Acetyl-Lactosamine ligand for the Ashwell Morell Receptor

Grewal PK et al. Nat Medicine 2008

The Ashwell–Morell receptor (AMR) of hepatocytes

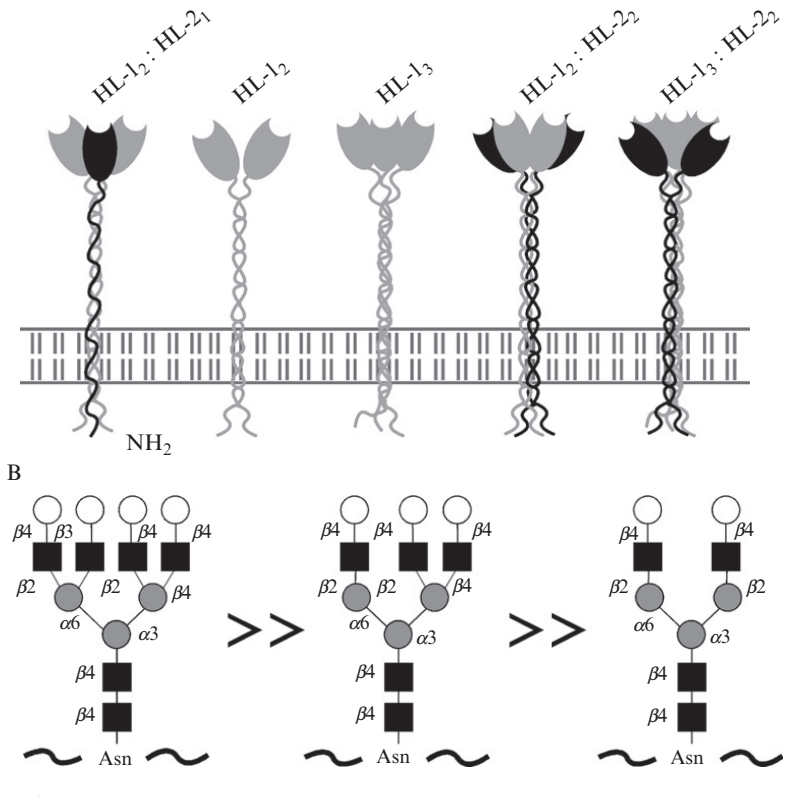


https://www.amgenscience.com/features/a-gene-linked-to-lower-risks-of-heart-attacks/

- ✓ Prototype C-type lectin
- ✓ Aswell and Morell 1960 and 1970
- ✓ ^{125}I-Sialylated proteins circulate after injection into circulation
- ✓ Rapid clearance of ^{125}I-asialylated glycoprotein\
- ✓ Removal of galactose prolonged life-time
- ✓ Radioactive protein sequestered in liver
- ✓ Ca^{2+} -dependent receptor purified from rabbit liver membranes

- ✓ Ashwell Receptor is composed of 2 glycoprotein receptor subunits:
  - ✓ Asialoglycoprotein receptor-1 (Asgr-1 or HL1)
  - ✓ Asiologlycoprotein receptor-2 (Asgr-2 or HL2)
  - ✓ ***The human AMR is a hetero-tetramer (Asgr1 : Asgr2 in a 3:1 ratio);***
- ✓ Mice deficient in either glycoprotein have diminished exogenous desialylated glycoproteins, but endogenous desialylated glycoproteins do not accumulate in animals lacking the receptor;
- ✓ Thus, ASGPR may be required to regulate serum glycoprotein levels under stress conditions which often elevates serum glycoprotein levels

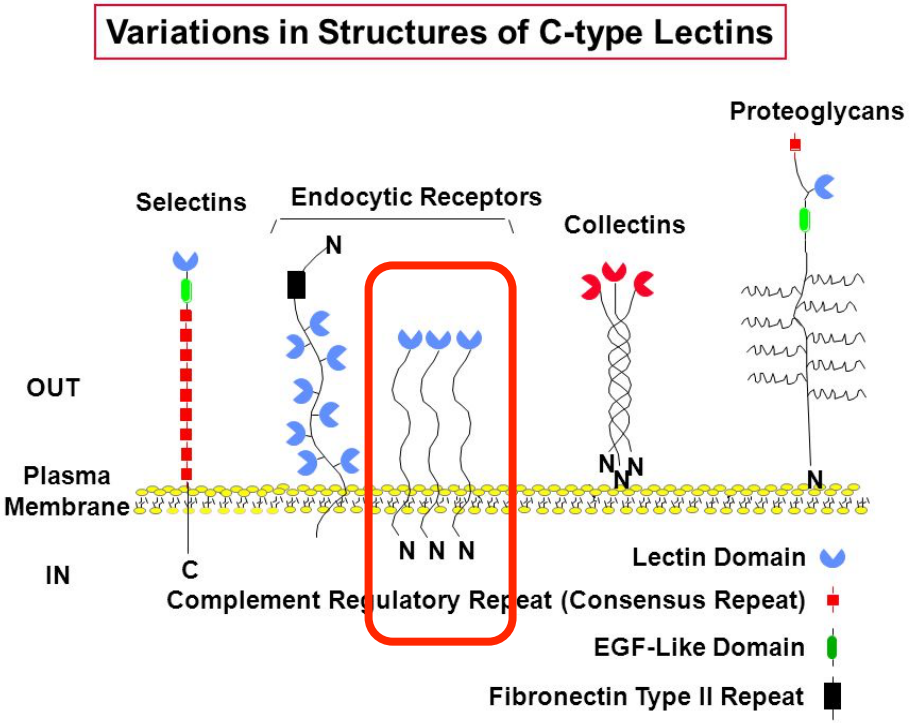
The Ashwell–Morell receptor (AMR) of hepatocytes



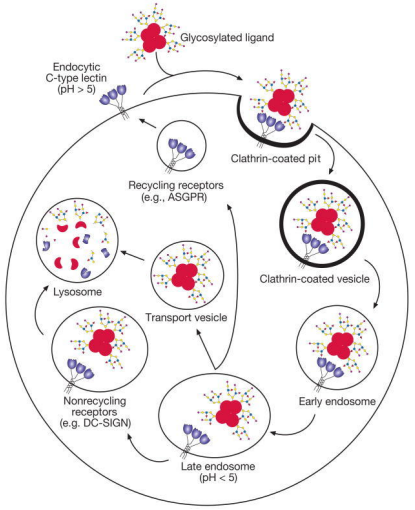
- The hepatic AMR forms Asgr1/Asgr2 homo- or hetero-dimers
- Dimer/trimer/tetramer formation is species dependent
- Tri- and tetra-antennary N-glycans with appropriate branching and presentation of non-reducing terminal galactose or N-acetyl-galactosamine residues bind to the rat ASGPR with greater than 100,000 times higher affinity (~nM range) than ligands with a single terminal Gal/GalNAc residue.

Grewal P. Methods in Enzymology, Vol. 479, 2010, p.223-241

C-type lectins



- ✓ C-type lectins come in different forms;
- ✓ The C-type lectin fold has been found in more than 1000 proteins;
- ✓ Glycan binding by the C-type lectins is always Ca^{2+}-dependent because of specific amino acid residues that coordinate Ca^{2+} and bind the hydroxyl groups of sugars
- ✓ The C-type lectin fold is an evolutionarily ancient structure that is adaptable for many uses.

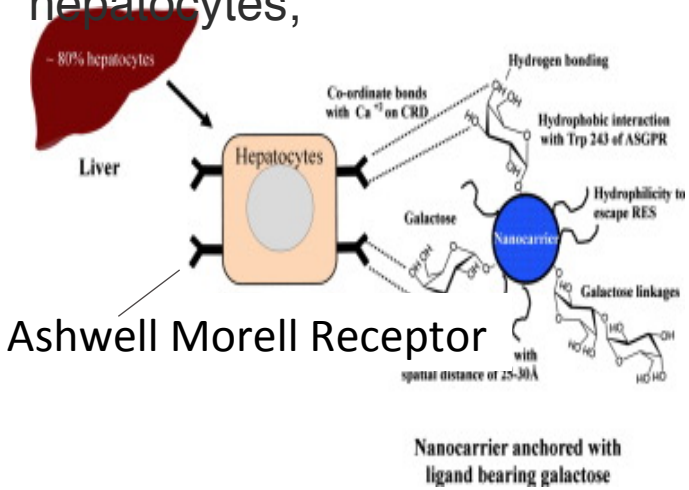


- ✓ The Ashwell receptor is an endocytic receptor
- ✓ Ligands are internalized by clathrin-dependent pathways and delivered to early and then late endosomes.
- ✓ Receptors may be recycled or degraded, depending on the receptor and the type of ligand it endocytoses

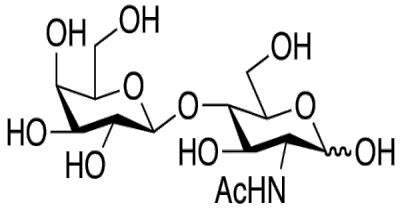
Essentials in Glycobiology, Chapter 31 C-type Lectins
Richard D Cummings and Rodger P McEver.

Ashwell Morell Receptor (AMR) is often used for hepatocyte targeted drug delivery

- ✓ AMR is expressed primarily in the liver;
- ✓ AMR is used as a marker for hepatocytes;



Nanocarrier anchored with ligand bearing galactose



N-Acetyl-Lactosamine ligand for the Ashwell Morell Receptor

Can we use this approach to design a TPO memetic?

https://doi.org/10.1016/j.jconrel.2015.02.022

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Variant *ASGR1* Associated with a Reduced Risk of Coronary Artery Disease

P. Nioi, A. Sigurdsson, G. Thorleifsson, H. Helgason, A.B. Agustsdottir, G.L. Norddahl, A. Helgadottir, A. Magnusdottir, A. Jonasdottir, S. Gretarsdottir, I. Jonsdottir, V. Steinthorsdottir, T. Rafnar, D.W. Swinkels, T.E. Galesloot, N. Grarup, T. Jørgensen, H. Vestergaard, T. Hansen, T. Lauritzen, A. Linneberg, N. Friedrich, N.T. Krarup, M. Fenger, U. Abildgaard, P.R. Hansen, A.M. Galløe, P.S. Braund, C.P. Nelson, A.S. Hall, M.J.A. Williams, A.M. van Rij, G.T. Jones, R.S. Patel, A.I. Levey, S. Hayek, S.H. Shah, M. Reilly, G.I. Eyjolfsson, O. Sigurdardottir, I. Olafsson, L.A. Kiemeney, A.A. Quyyumi, D.J. Rader, W.E. Kraus, N.J. Samani, O. Pedersen, G. Thorgeirsson, G. Masson, H. Holm, D. Gudbjartsson, P. Sulem, U. Thorsteinsdottir, and K. Stefansson

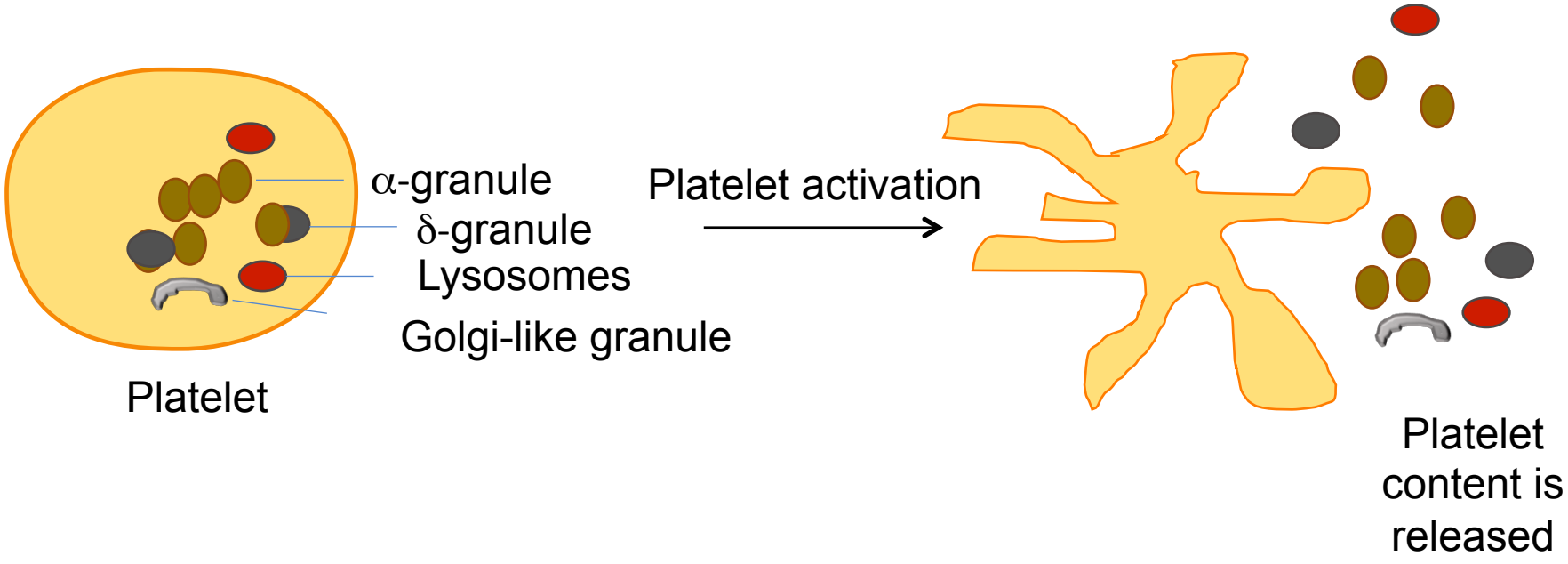
1. Nioi, P, et al. *New Eng J Med.* 2016;374(22): 2131-2141.

2. Tybjærg-Hansen, A. *New Eng J Med.* 2016;374(22):2169-2171.

ASGR1 Variants discovered by AMGEN-science

- ✓ Sequenced genomes of 2636 Icelanders and found variants that were then imputed into the genomes of approximately 398,000 Icelanders; deCODE population
- ✓ Found rare noncoding 12-base-pair (bp) deletion (del12) in intron 4 of *ASGR1;*
- ✓ Individuals Have *loss of AMR receptor function*
- ✓ Have lower non-HLD, LDL cholesterol
- ✓ Have increase in Alkaline Phosphatase
- ✓ "The cardioprotective effect is one of the biggest we've seen with any gene variant, and it's hard to explain this large effect based solely on the gene's impact on cholesterol levels." *https://www.amgenscience.com/items/amgens-asgr1-research-gene-x/*
- ✓ AMGEN developed inhibitor AMG529;
- ✓ Phase 1 clinical trial initiated in 2017

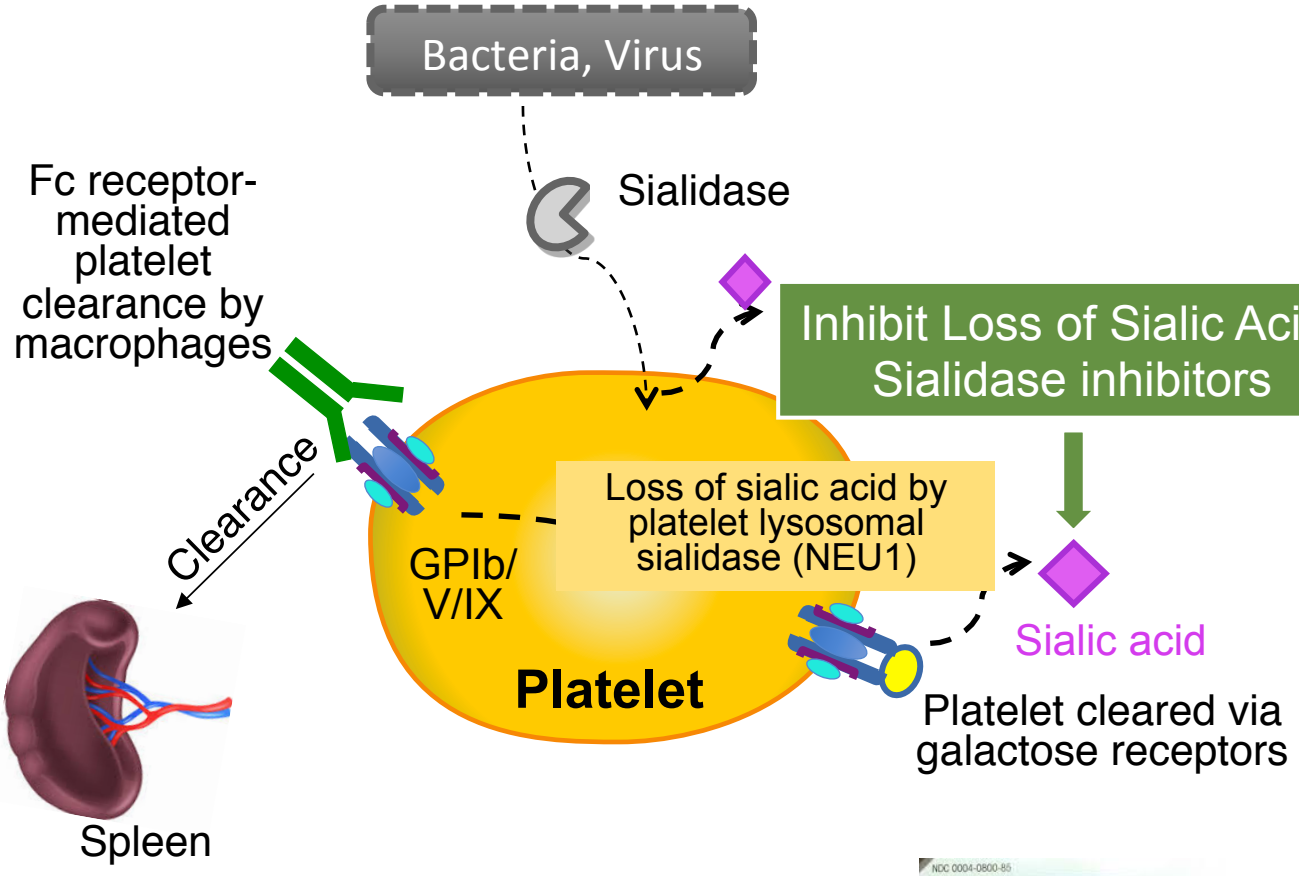
Platelet biology



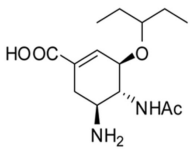
Platelet contain 4 forms of granule:

- ✓ α-granule: most abundant, majority of platelet factors involved in hemostasis and thrombosis: thrombospondin, P-selectin, platelet factor 4 and beta thromboglobulins coagulation factors V, XI, XIII, fibrinogen, von Willebrand factor and high molecular weight kininogens, cytokines and such as PDGF etc.
- ✓ δ-granule: smallest granules, high calcium and phosphate content; contain high concentrations of adenine nucleotides and serotonin.
- ✓ *Lysosomes: contain Sialidase (NEU1), β-galactosidase, b; released upon activation, may contribute to regulation of thrombus formation and remodeling of the extracellular matrix*
- ✓ Golgi-like granule: activated sugar nucleotide donors, glycosyl transferases

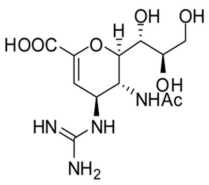
Translational Perspectives of "Platelet" Glycobiology: Thrombocytopenias



Chemical structures of influenza virus sialidase inhibitors.



Oseltamivir carboxylate



Zanamivir

- ❑ **FDA approved inhibitors developed for influenza virus sialidases:**
- ✓ Zanamivir (Relenza), administered by inhalation;
- ✓ Oseltamivir (Tamiflu), administered orally;
- ✓ Laninamivir in phase III clinical trials;
- ✓ Zanamavir selectively inhibits neuraminidase of Influenza A and B;
- ✓ In a population of patients Tamiflu significantly improved thrombocytopenia;
- ✓ Thrombocytopenia in patients with sepsis can be ameliorated by Tamiflu administration

Translational Perspectives of “Platelet” Glycobiology: Thrombocytopenias: Tamiflu-ITP trial

Study Type : Interventional (Clinical Trial)
Estimated Enrollment : 30 participants
Allocation: Randomized
Intervention Model: Parallel Assignment
Masking: Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor)
Primary Purpose: Treatment
Official Title: Proof of Concept; A Pilot, Randomized, Double-Blind Study of Oseltamivir Versus Placebo for Immune Thrombocytopenia
Actual Study Start Date : November 2016
Estimated Primary Completion Date : January 2019
Estimated Study Completion Date : November 2019