Thrombosis

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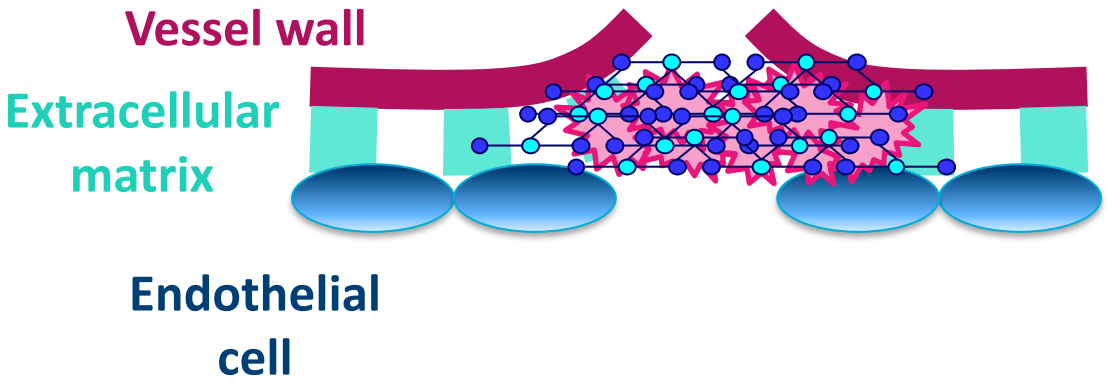
Thrombosis : physiopathology

Thrombus = bleeding cessation



Physiological hemostasis

- **Primary hemostasis = Platelet plug**
- **Coagulation = Fibrin network**

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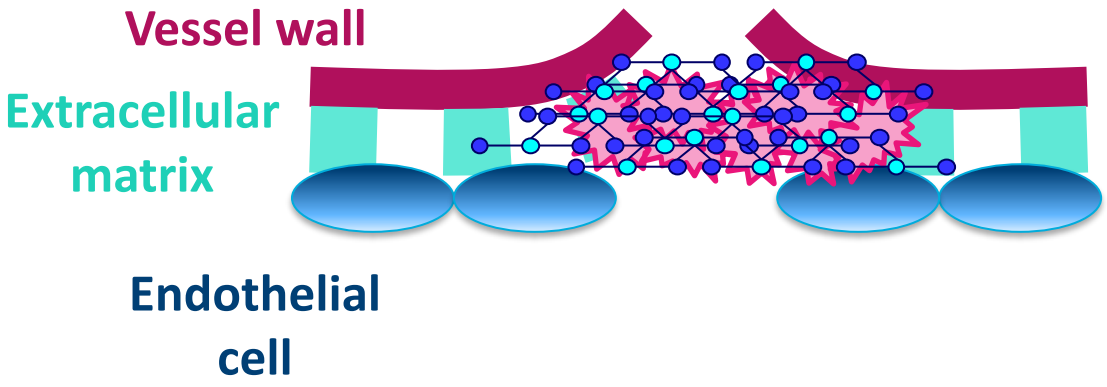

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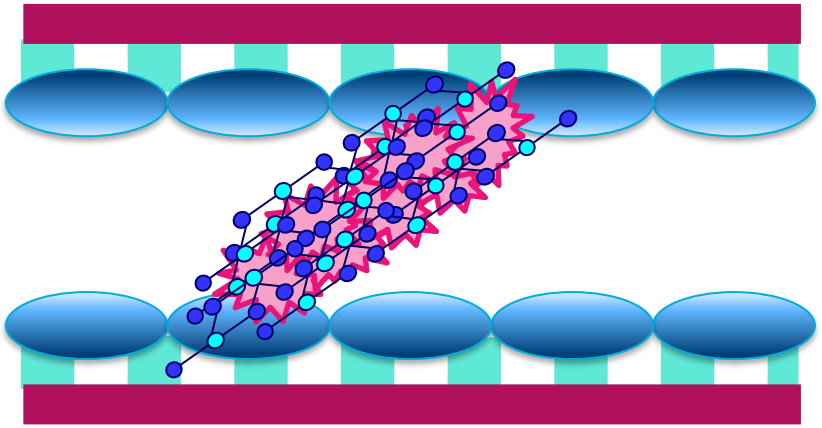
Thrombus = blood flow obstruction ⇨ hypoperfusion ⇨ ischemia



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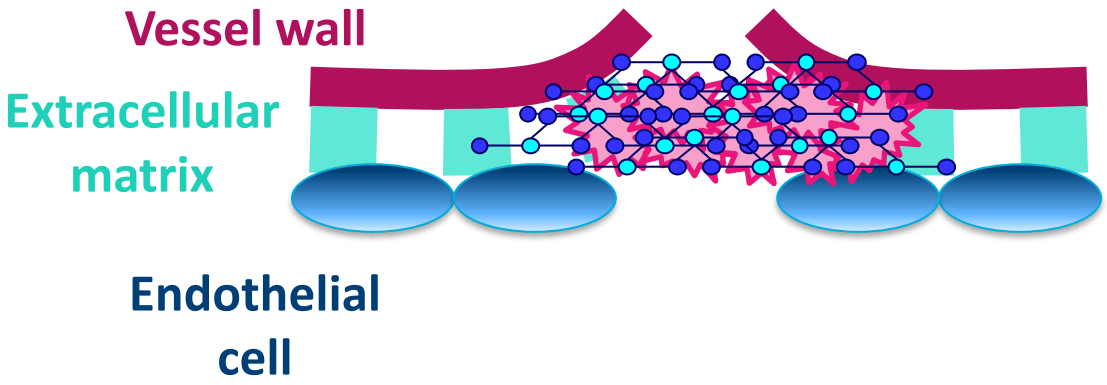

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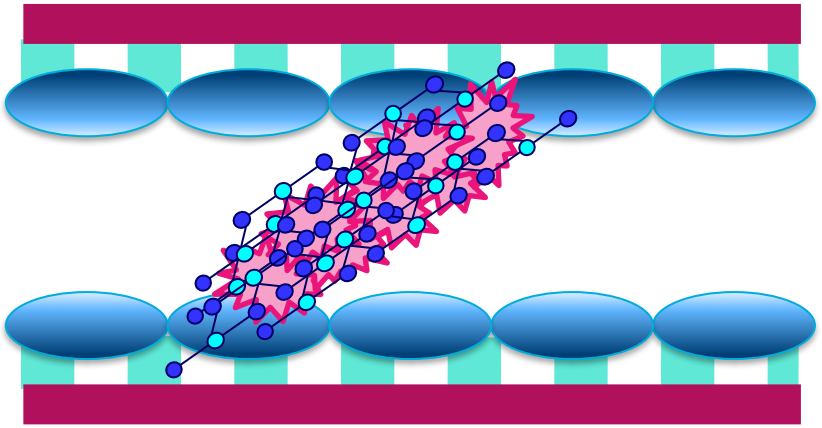


Pathological thrombosis

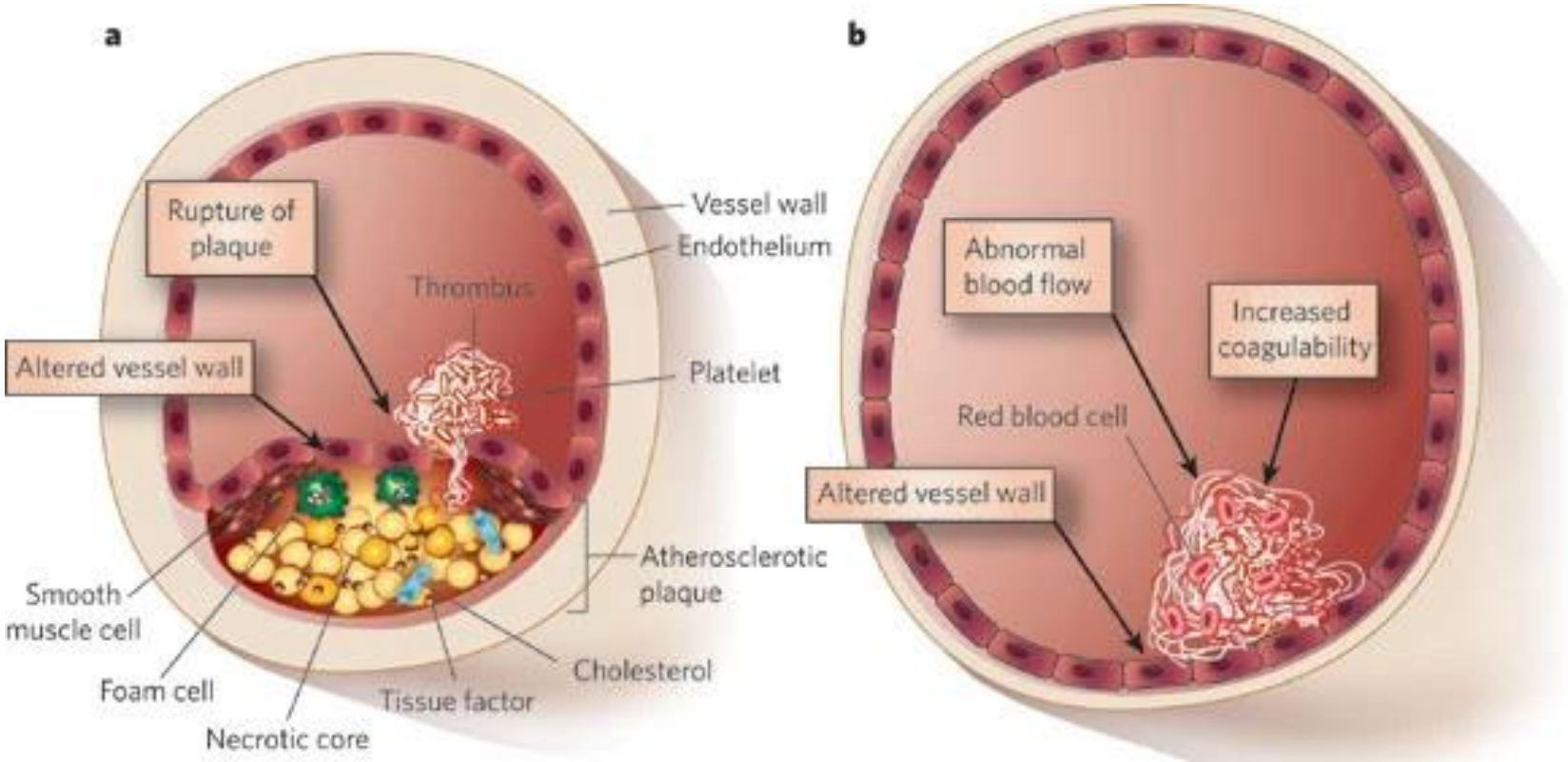
- **Primary hemostasis = Platelet plug**

  Antiplatelet drugs

- **Coagulation = Fibrin network**

  Anticoagulant drugs

Thrombosis : physiopathology



Arterial thrombosis

Damaged vessel wall: Atherosclerosis

High shear forces

Platelet-rich thrombus

⬇

Antiplatelet drugs

Venous thrombosis

Not associated with atherosclerosis

Low shear forces

Fibrin-rich thrombus

⬇

Anticoagulant drugs

Thrombosis : diagnosis

Physical exam

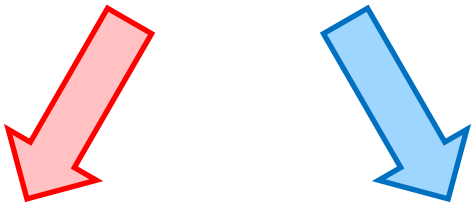
- **Medical history/Clinical context**
- **Visible signs of thrombosis (swelling, tissue or temperature changes)**
- **Heart pulse, blood pressure**

Imaging tests

- **X-rays of blood vessels (angiogram or venogram)**
- **Ultrasound (Doppler)**
- **Magnetic resonance imaging (MRI).**

Blood tests

- **Platelets count**
- **D-Dimer**



Arterial thrombosis **Venous thrombosis**

Thrombosis : diagnosis

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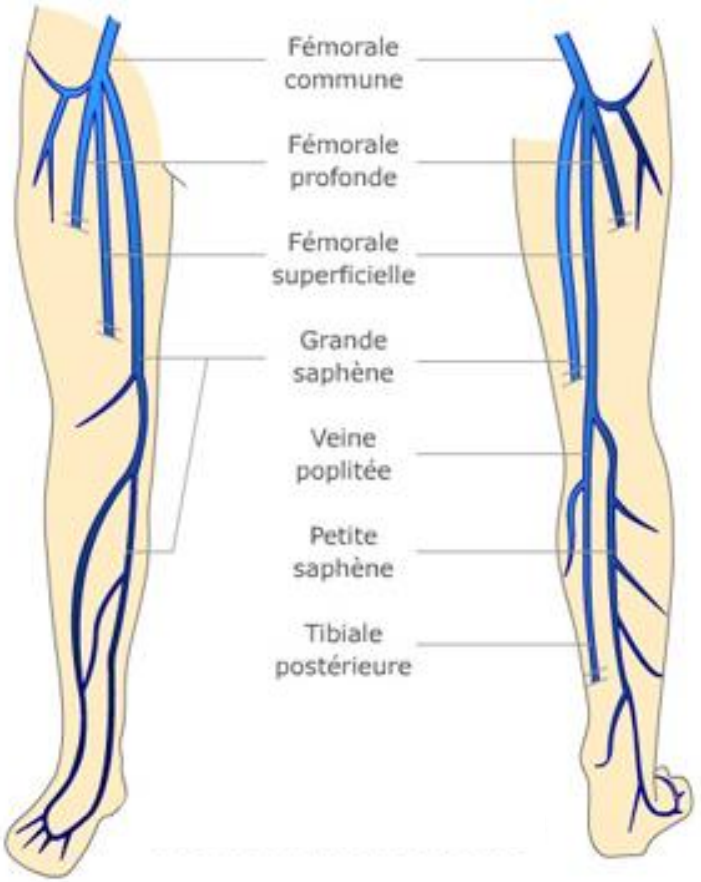
Arterial thrombosis **Venous thrombosis**

Venous thrombosis

venous thromboembolic disease (VTE)



Proximal DVT

Distal DVT

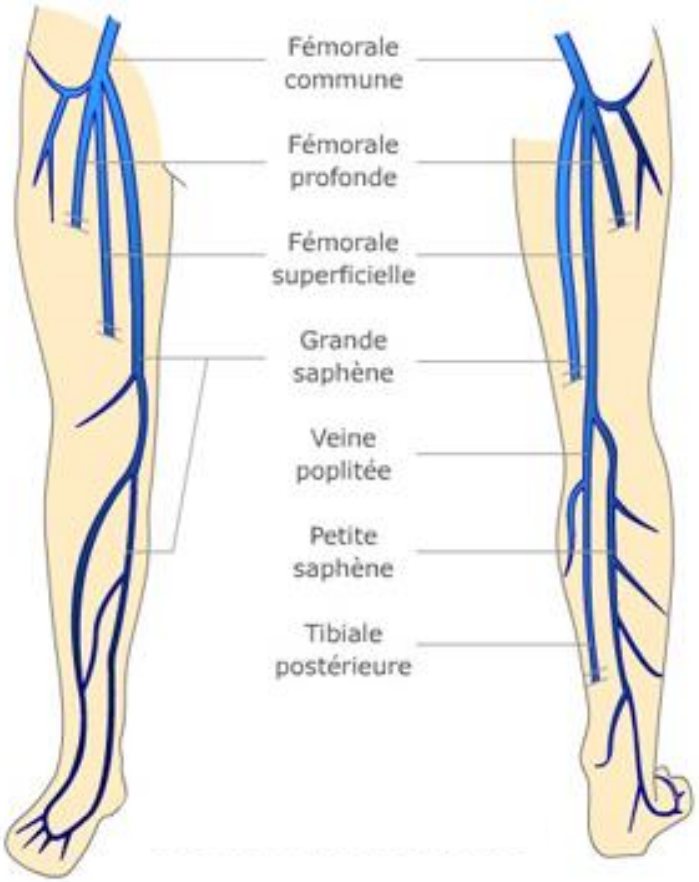
Symptoms :

- Leg swelling
- Leg pain, cramping or soreness that often starts in the calf
- Change in skin color on the leg (red or purple)
- A feeling of warmth on the affected leg

Deep vein thrombosis (DVT)

Venous thrombosis

venous thromboembolic disease (VTE)



Proximal DVT

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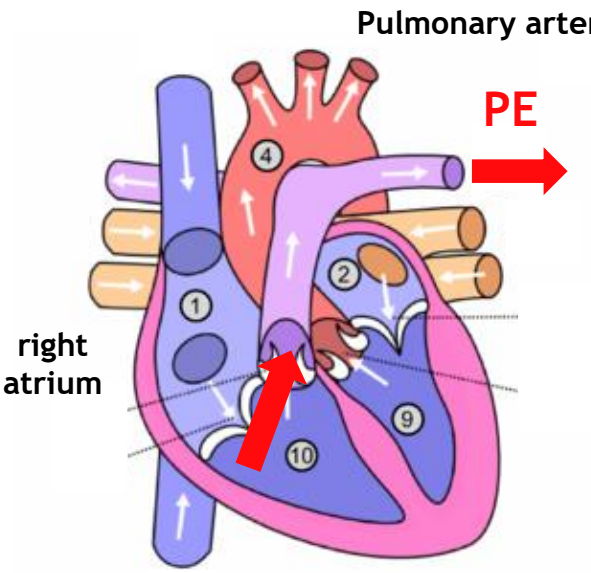
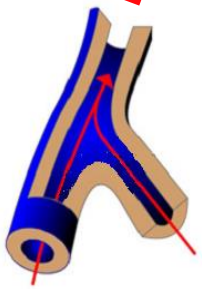


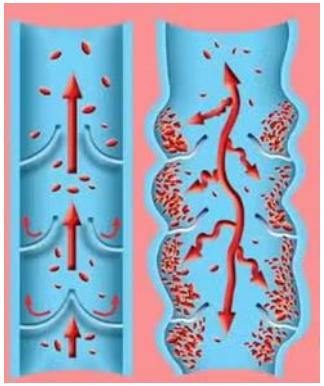


PE = Life-threatening complication of DVT

Deep vein thrombosis (DVT)

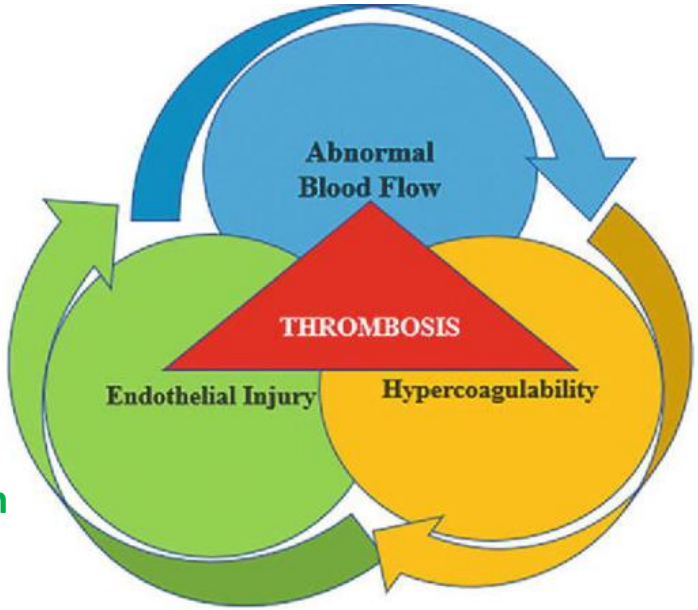
Pulmonary embolism (PE)

Venous thrombosis



Dilated vein ⇨ Damaged/deformed Valves
Aging
Immobilization
Compression

⇨ **Blood stasis**



Surgery, trauma
Cancer, inflammation
Oxidative stress (aging, smoking, obesity, …)

⇨ **Alteration of the endothelium**

Anticoagulant factor deficiency (AT, PC, PS, TFPI)
Excess of procoagulant factors (FV Leiden, FII G20210A)
Antiphospholipids syndrom

⇨ **Hypercoagulability**

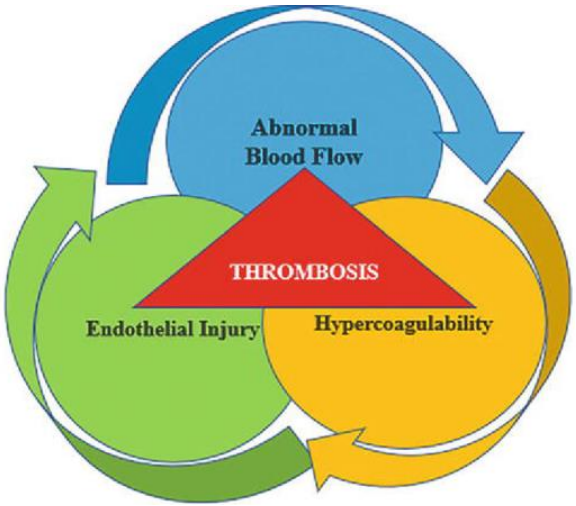
Virchow's triad (first described by Dr Virchow in 1858, and still relevant!)

Venous thrombosis

Risk Factors for Thromboembolism disease

Acquired

- Surgery
- Age
- Immobilization
  - Travel
  - Hospitalization
  - …..
- Obesity
- Cancer
- Pregnancy
- Hormonal treatment
- ….



Genetic

- Deficit in AT, PC, PS
- FV Leiden
- FII Mutation

Venous thrombosis

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- Surgery
- Age
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  - …..
- Obesity
- Cancer
- Pregnancy
- Hormonal treatment
- ….

Genetic

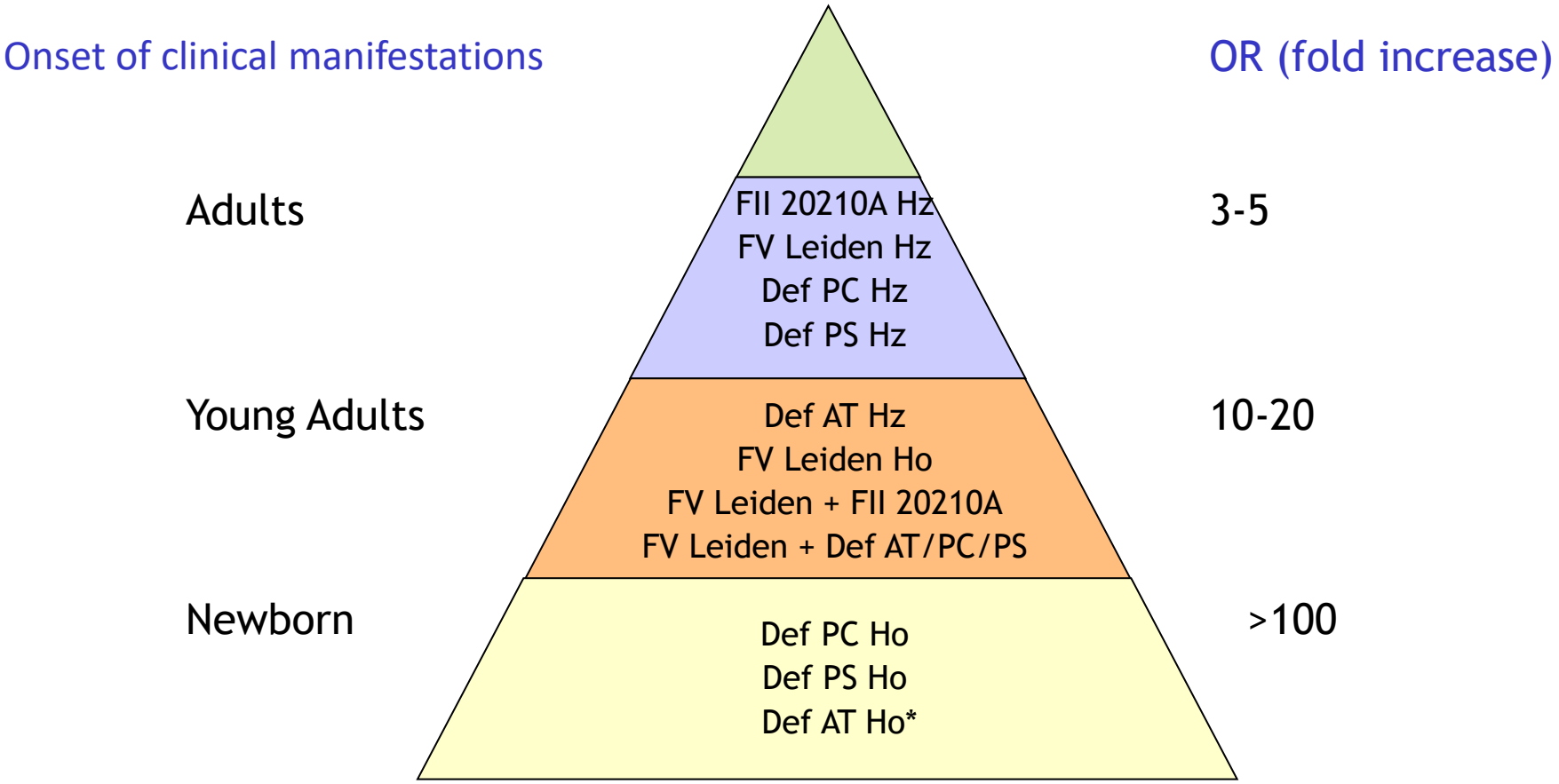
- Deficit in AT, PC, PS
- FV Leiden
- FII Mutation

Examples:

Casts of the lower limbs	Odd ratio **36**
Orthopedic Surgery	OR **16**
Immobilization	OR **7**
Long travel	OR **1.6**

Venous thrombosis

Risk associated with biological thrombophilia



DVT diagnosis

Clinical score for DVT

Table 2. Simplified Clinical Model for Assessment of Deep Vein Thrombosis*

Clinical Variable	Score
Active cancer (treatment ongoing or within previous 6 months or palliative)	1
Paralysis, paresis, or recent plaster immobilization of the lower extremities	1
Recently bedridden for 3 days or more, or major surgery within the previous 12 weeks requiring general or regional anesthesia	1
Localized tenderness along the distribution of the deep venous system	1
Entire leg swelling	1
Calf swelling at least 3 cm larger than that on the asymptomatic leg (measured 10 cm below the tibial tuberosity)†	1
Pitting edema confined to the symptomatic leg	1
Collateral superficial veins (nonvaricose)	1
Previously documented DVT	1
Alternative diagnosis at least as likely as DVT	–2

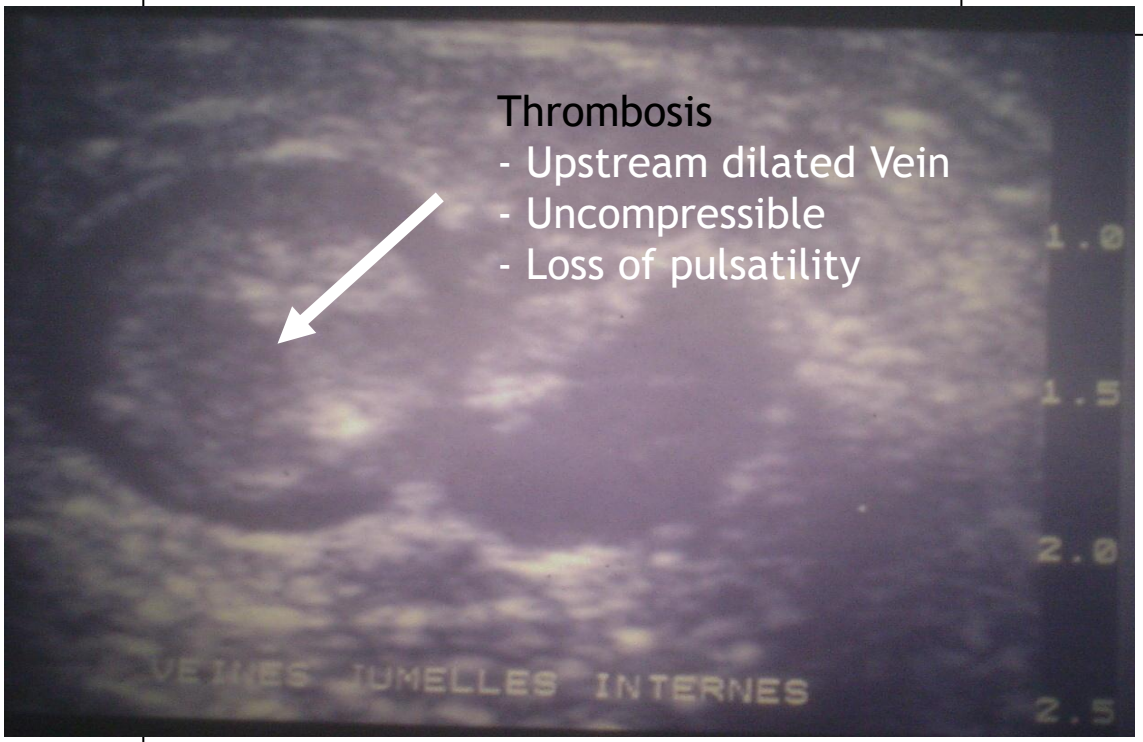
Weak Probability	≤ 0
Moderate Probability	1-2
High Probability	≥ 3

Wells PS JAMA 2006

DVT diagnosis

Clinical score for DVT

Evaluation of DVT clinical probability (Score)



High

Venous echo-Doppler

No DVT

DVT

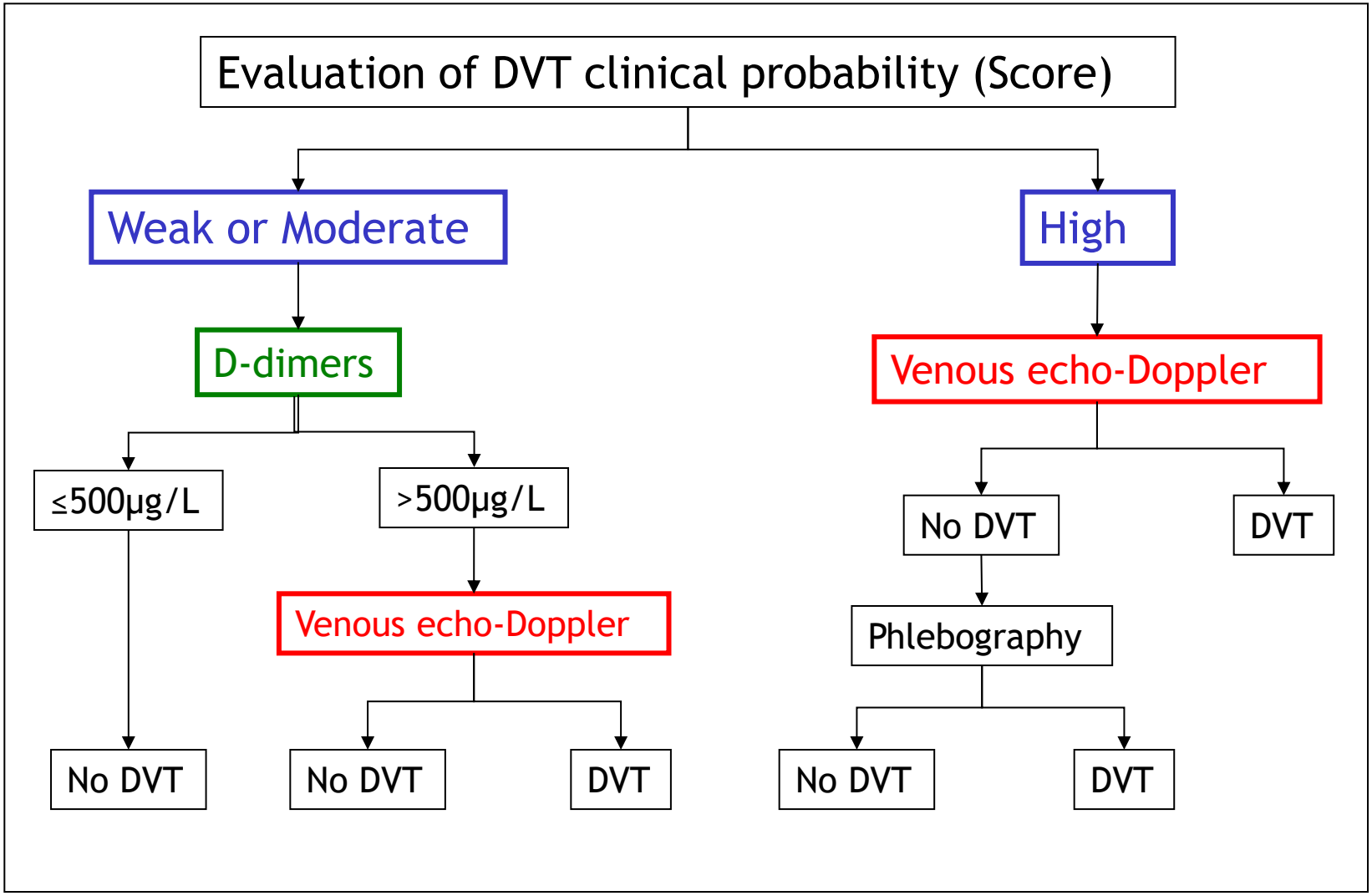
Phlebography

No DVT

DVT

DVT diagnosis

Clinical score for DVT



DVT diagnosis

When must we search for biological thrombophilia?

- Thrombosis in young subjects (< 45 years)
- Recurrent Thrombosis
- Thrombosis of unusual localization

- In particular if the DVT is spontaneous

- Family history of DVT

DVT treatments

Anticoagulants to act on coagulation !

They don't "remove" an existing clot, but they can prevent it from growing

PE may require thrombolytic therapy (fibrinolysis)

Emergency treatment for life-threatening PE

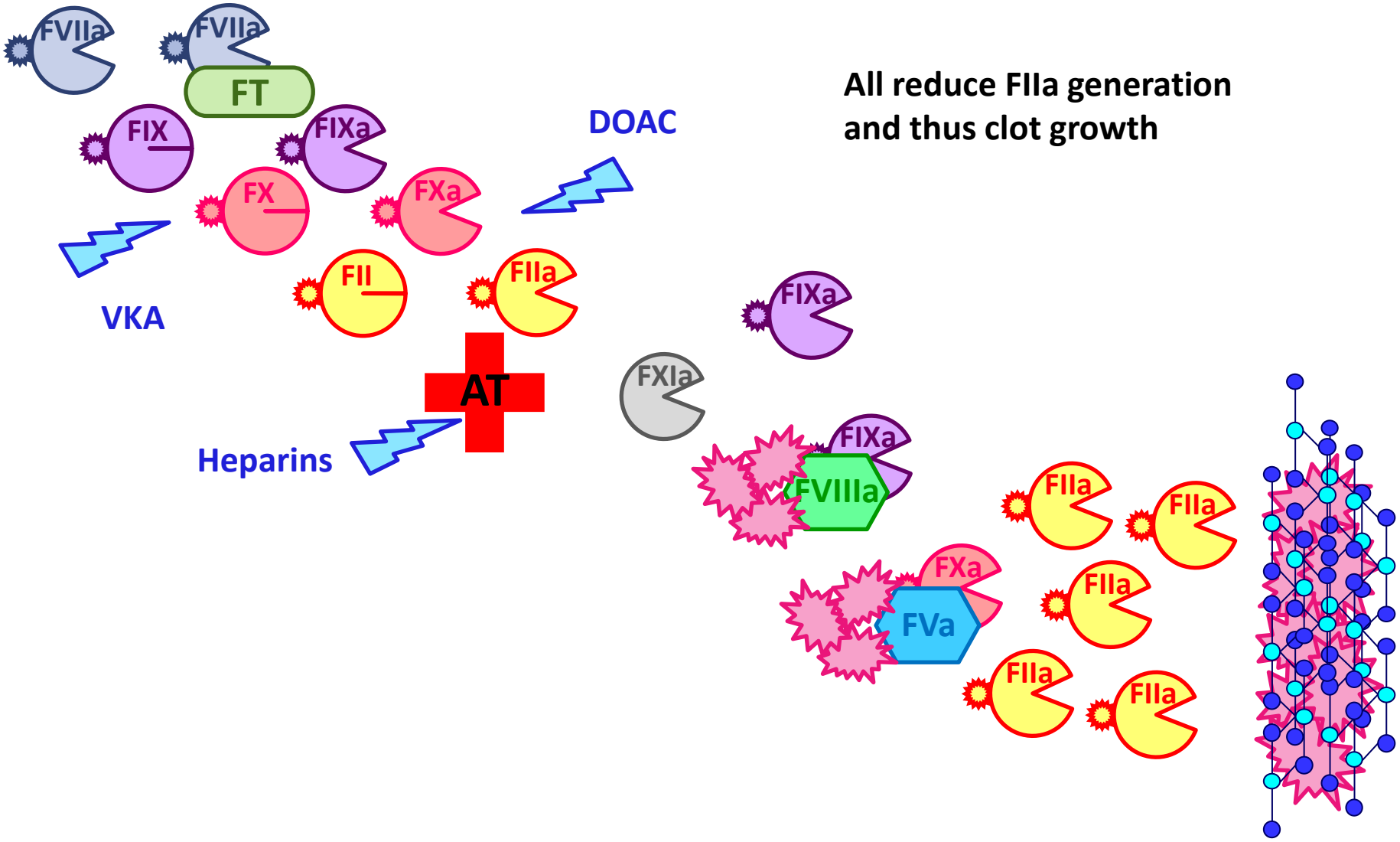
3 therapeutic classes :

Heparins (IV or SC)

VKA = Vitamin K Antagonists (oral)

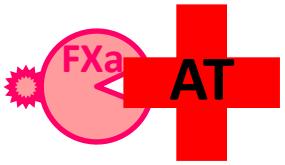
DOAC = Direct Oral AntiCoagulants (oral)

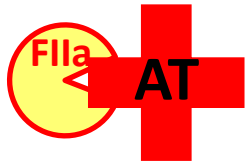
Anticoagulants



All reduce FIIa generation and thus clot growth

Anticoagulants





Heparins (IV or SC)

Sulfated polysaccharides

Bind to Antithrombin

Potentiate its anti-FIIa/FXa activity

Unfractionated heparin (UFH)

Long chains

Mean MW = 14-18 kD

Low molecular weigh heparin (LMWH)

Shorter chains

Mean MW ~ 4 kD

Animal products

Fondaparinux (Arixtra®)

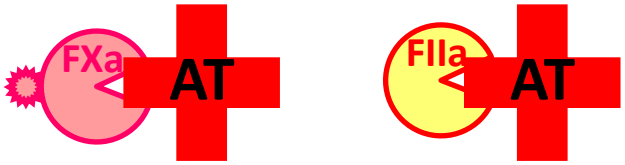
The shortest chain able to bind AT

MW = 1,7 kD

Chemical product

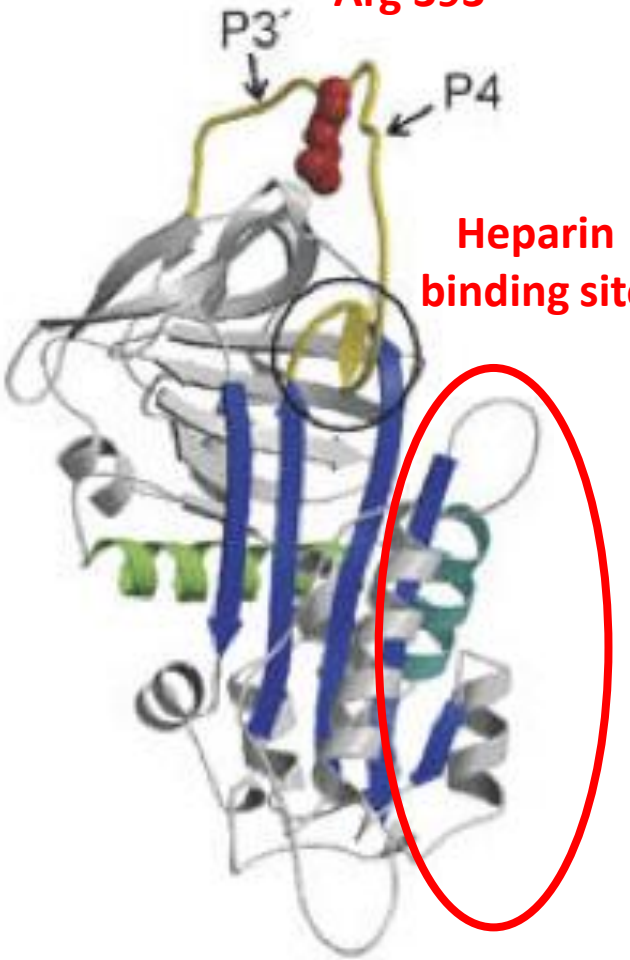
Anticoagulants

Heparins : mechanism of action



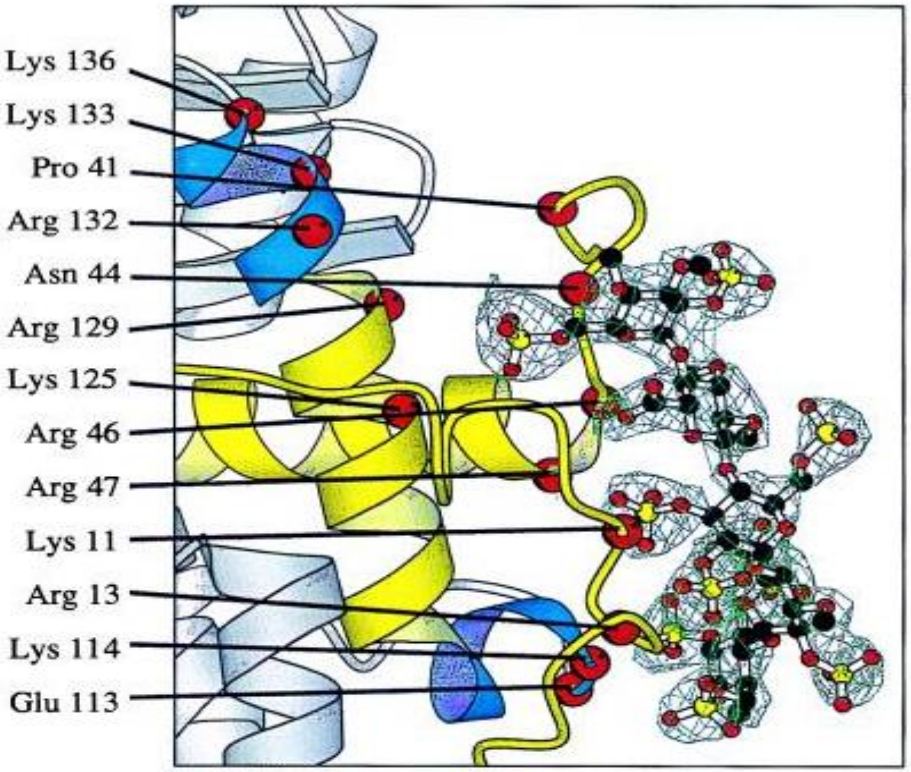
Active site

Arg 393



Heparin binding site

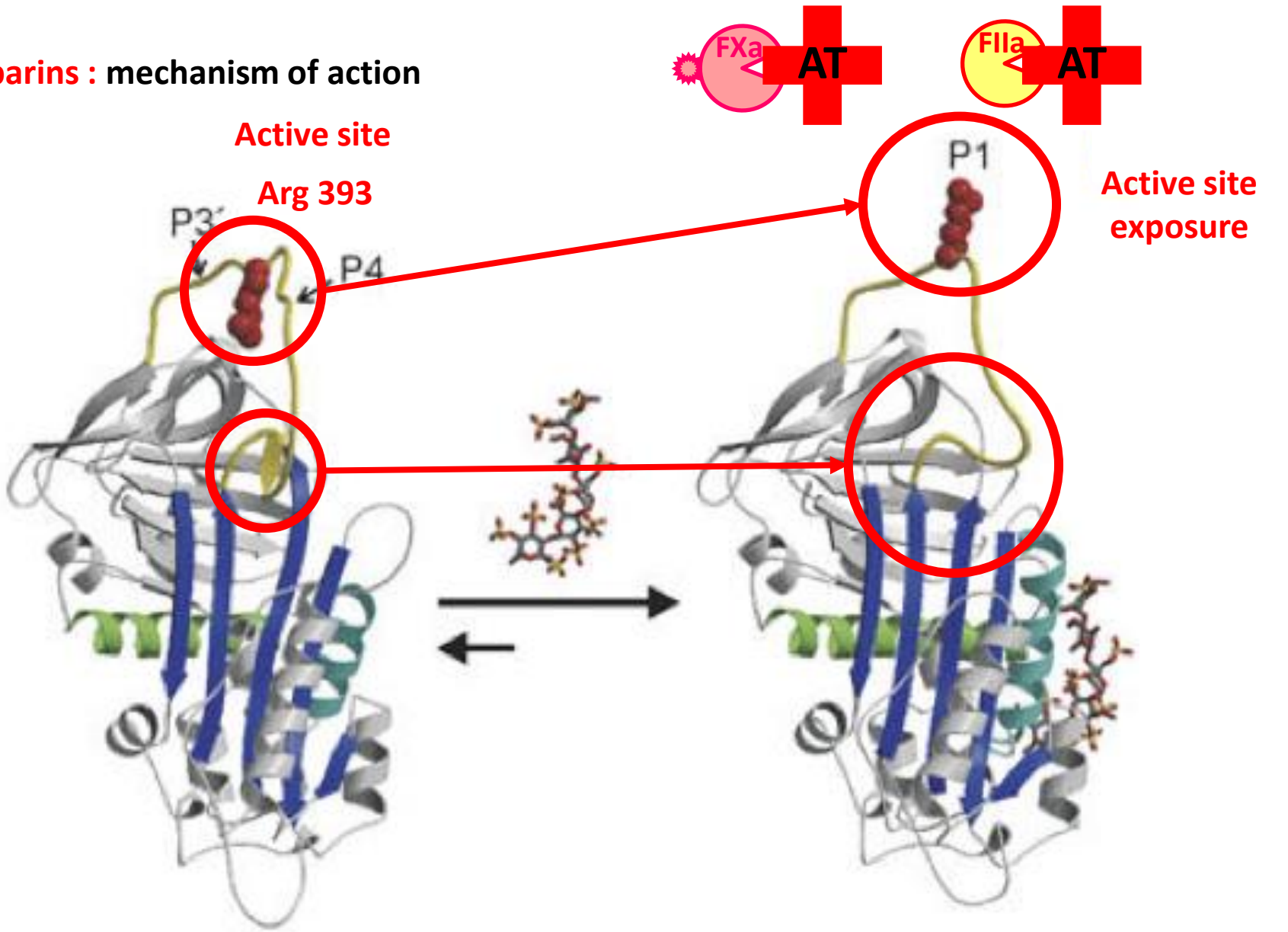
NH^+ **SO_3^-**



Several ionic interactions

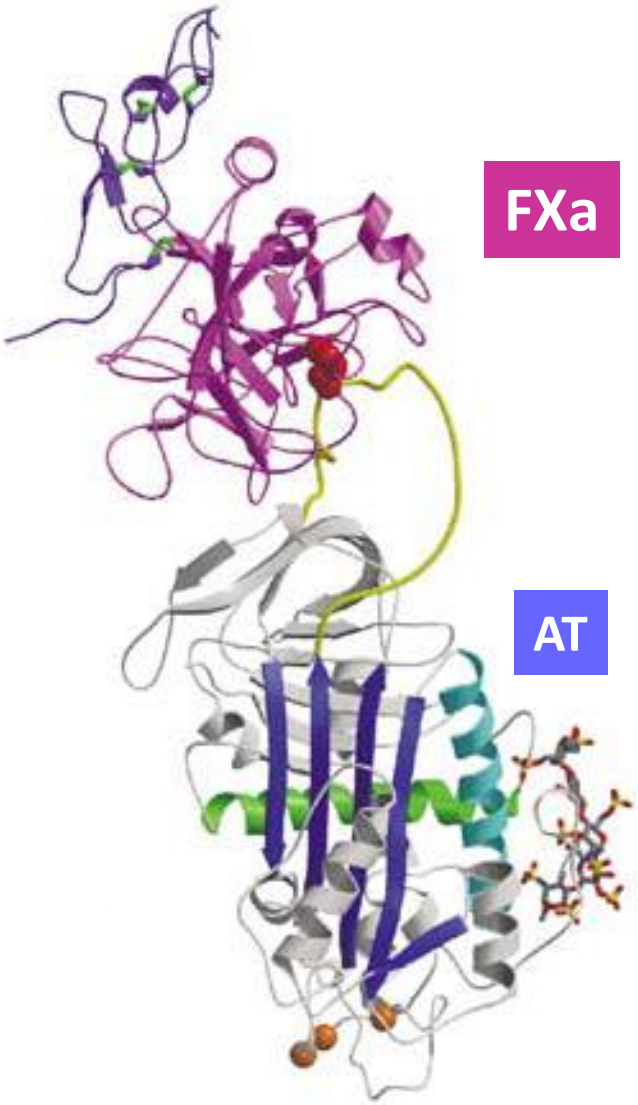
Anticoagulants

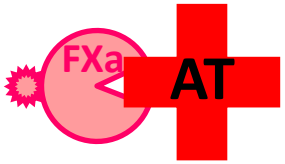
Heparins : mechanism of action



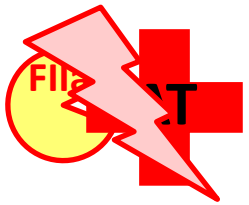
Anticoagulants

Heparins : mechanism of action





Increased reactivity toward FXa
Interaction 300-fold faster

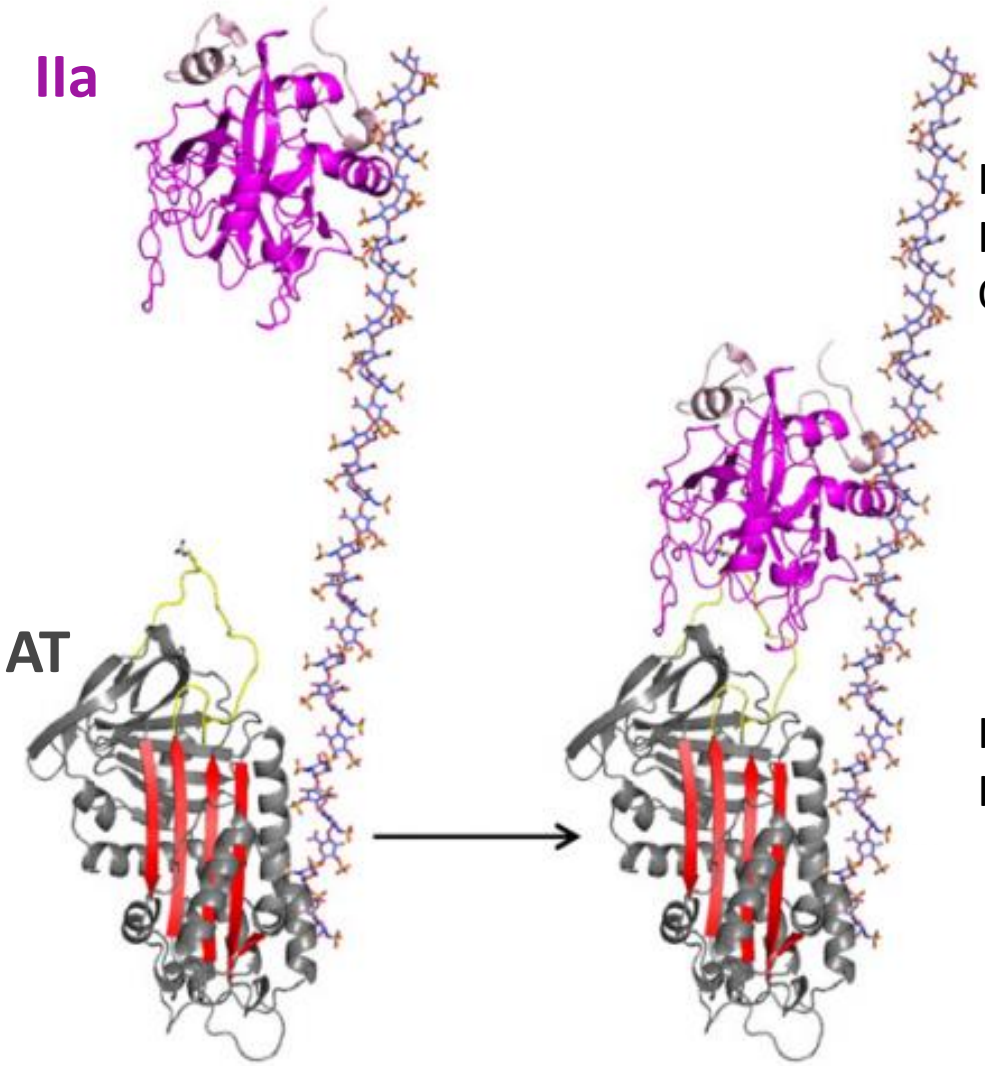


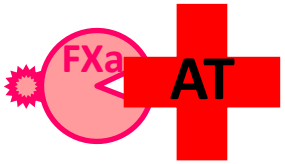
No effect on FIIa/AT interaction
Allosteric activation is not sufficient to accelerate FIIa-AT complex formation

Allosteric mechanism

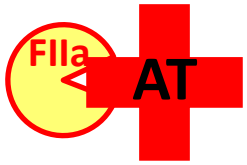
Anticoagulants

Heparins : mechanism of action





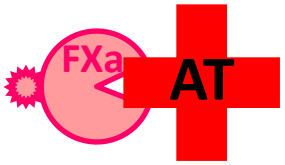
Increased reactivity toward FXa
Interaction 600-fold faster
Only 2-fold faster than with short chain

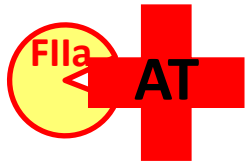


Increased reactivity toward FXa
Interaction 4,000-fold faster

Allosteric + bridging mechanism

Anticoagulants





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Bind to Antithrombin

Potentiate its anti-FIIa/Fxa activity

Unfractionated heparin (UFH)

Long chains

Mean MW = 14-18 kD

Both anti-FXa and FIIa effect

Low molecular weigh heparin (LMWH)

Shorter chains

Mean MW ~ 4 kD

Mainly anti-FXa effect

Fondaparinux (Arixtra®)

The shortest chain able to bind AT

MW = 1,7 kD

Only anti-FXa effect

Anticoagulants

Heparins (IV or SC)

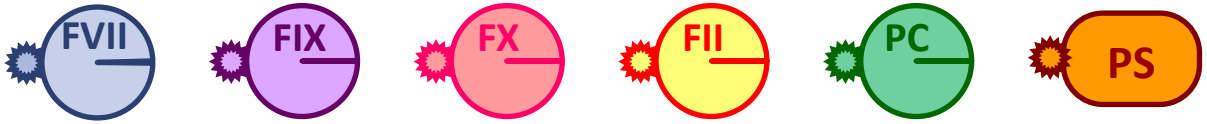
	UFH	LMWH	Fondaparinux
$t_{1/2}$	Short 1-2 hrs	4 hrs	15h
Biodisponibility	Low ~ 10% High interindividual variability	> 90% Low interindividual variability	100% No interindividual variability
Monitoring	APTT mandatory	Not required only if renal failure, extreme weight, hemorrhage	Not required only if renal failure, extreme weight, hemorrhage
Elimination	Cellular + kidney	Mainly kidney	Kidney only
Limitation	Heparin-induced thrombocytopenia	Heparin-induced thrombocytopenia	**No antidote !**

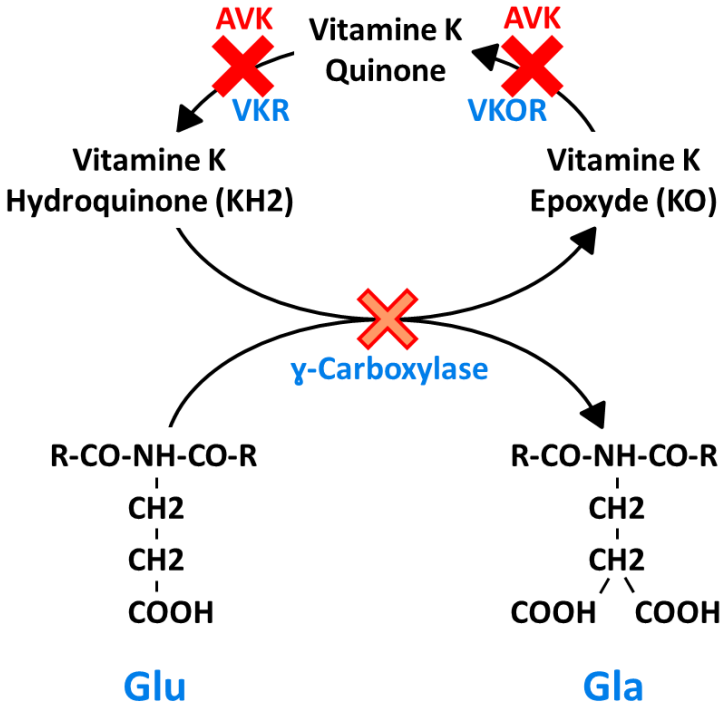
Anticoagulants

VKA = Vitamin K antagonists (oral route)

Vit K required for functional Gla domain synthesis

VKA impair synthesis of vit K-dependent foctors





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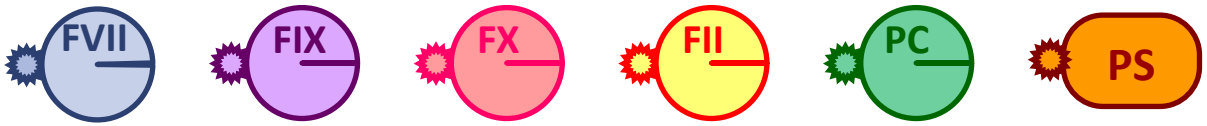


Coumarins (warfarin, acenocoumarol) + ~~fluindion~~

Delay of action (due to their mechanism of action) = 3-4 days

CYP-dependent complex metabolism ⇨ several food and drug interactions

High interindividual variability

Monitoring required = INR (international normalized ratio PT patient/PT normal plasma)

Target INR (DVT or PE prevention) = 2-3.

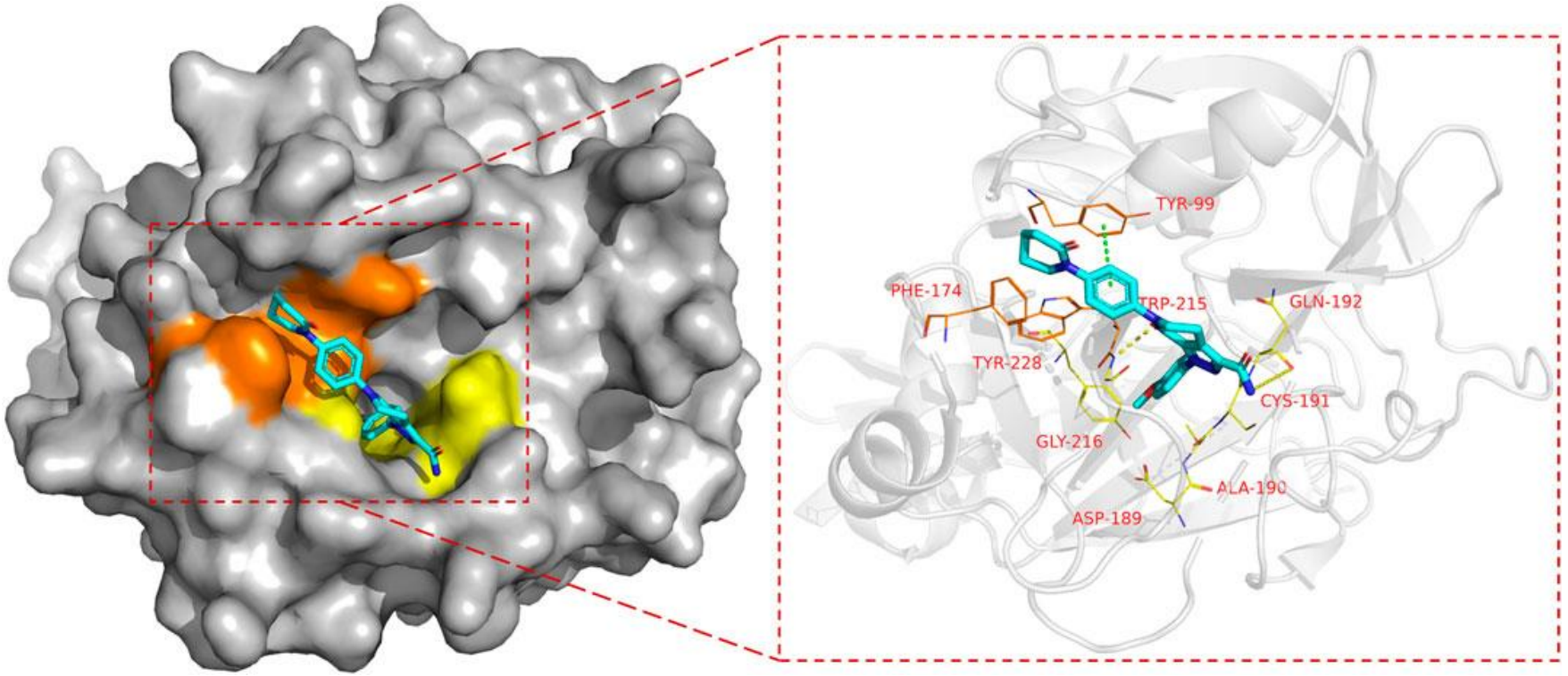
If INR < 2 inadequate prevention. If > 3 bleeding risk !!

Reversal = Vit K-dpdt factor concentrate / Vit K

Anticoagulants

DOAC = Direct Oral AntiCoagulants (oral route)

Direct reversible and competitive enzyme inhibitors



Anticoagulants

DOAC = Direct Oral AntiCoagulants (oral route)

Direct reversible and competitive enzyme inhibitors

Dabigatran (Pradaxa®) ⇨ **target FIIa**

Rivaroxaban (Xarelto®)
Apixaban (Eliquis®)
Edoxaban (Lixiana®)
} ⇨ **target FXa** **"Xaban"**

Anticoagulants

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} ⇨ **target FXa** **"Xaban"**

Many assets over VKA, more and more prescribed

Short delay of action (t_{max} ~ 3 hrs)

Very few food and drug interactions

Low interindividual variability

But same risk of bleeding events

Renal elimination

No monitoring (except specific situations, renal failure, hemorrhage, invasive surgery)

INR not suitable for DOAC, anti-FXa or anti-FIIa activity

Anticoagulants

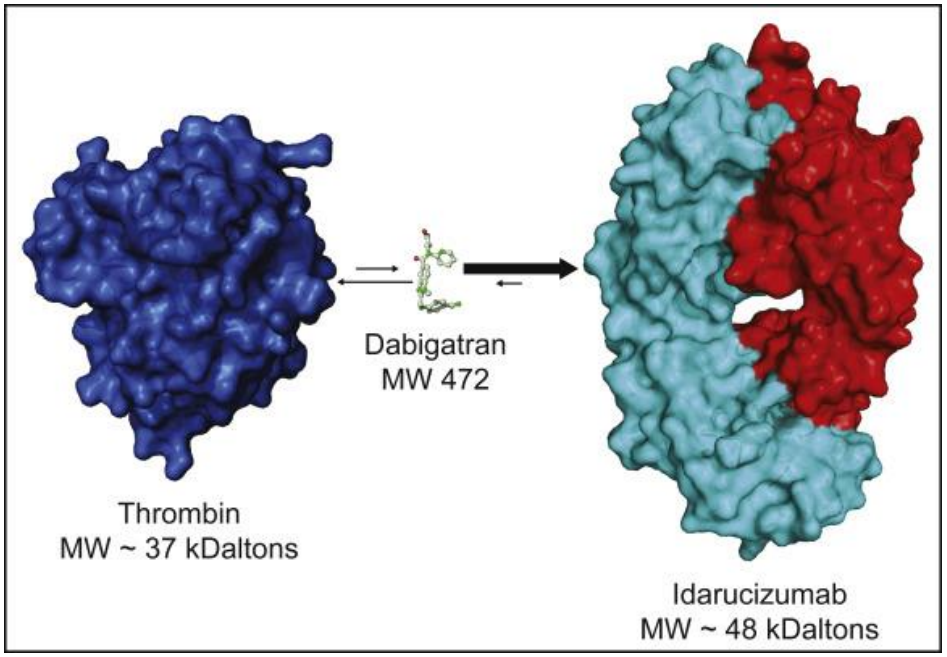
Need for a reversal agent

DOAC = Direct Oral AntiCoagulants (oral route)

Direct reversible and competitive enzyme inhibitors

Dabigatran (Pradaxa®) ⇨ **target FIIa**

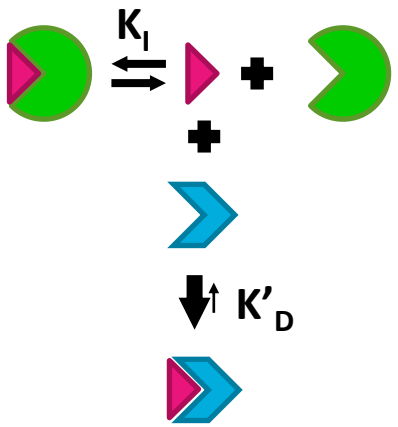
Idarucizumab (Paxbind®)



Humanized mAb, that diverts dabigatran from FIIa binding

K'_D for dabigatran ~2,1 pM
K_I (IIa/Dabigatran) ~ 4,5 nM

Almost irreversible !



Anticoagulants

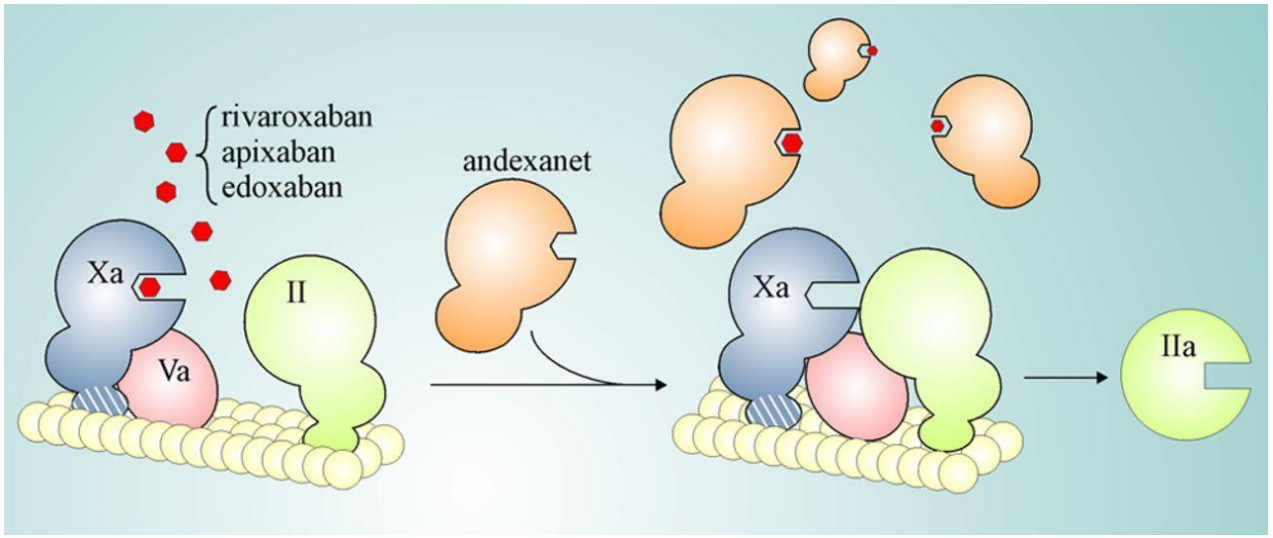
Need for a reversal agent

DOAC = Direct Oral AntiCoagulants (oral route)

Direct reversible and competitive enzyme inhibitors

Dabigatran (Pradaxa®) ⇨ **target FIIa** — **Idarucizumab (Paxbind®)**

Rivaroxaban (Xarelto®)
Apixaban (Eliquis®)
Edoxaban (Lixiana®)
} ⇨ **target FXa = "Xaban"** — **Andexanet-alpha (Ondexxya®)**



Recombinant inactive gla domain-less FXa

Anticoagulants

Indication	Drug
DVT/PE acute phase	Heparin or DOAC
DVT/PE prevention (depends on clinical context)	DOAC (or AVK) if long term Heparin if short term
Stroke prevention in patients with atrial fibrillation (AF)	Long term DOAC (VKA if ongoing treatment)
Kidney failure requiring dialysis	Heparin

Thrombosis : diagnosis

Physical exam

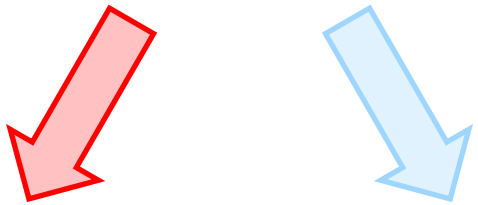
- **Medical history/Clinical context**
- **visible signs of thrombosis (swelling, tissue or temperature changes)**
- **Heart pulse, blood pressure**

Imaging tests

- **X-rays of blood vessels (angiogram or venogram)**
- **Ultrasound (Doppler)**
- **Magnetic resonance imaging (MRI).**

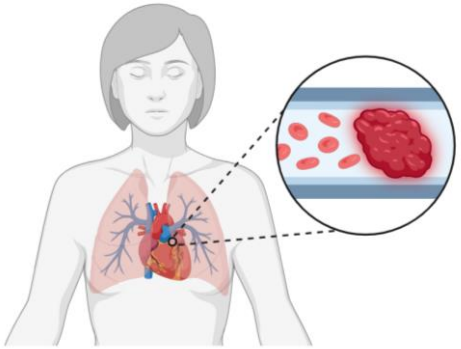
Blood tests

- **Platelets count**
- **D-Dimer**

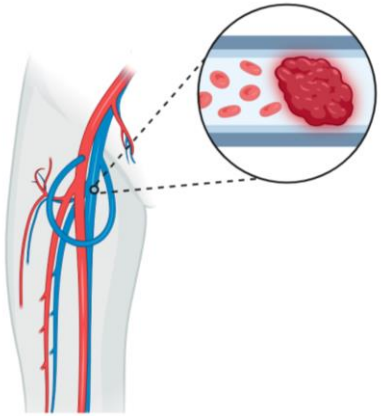


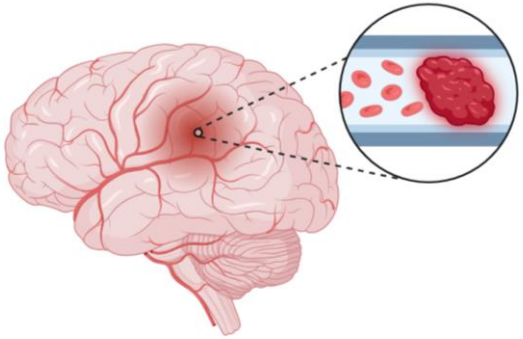
Arterial thrombosis **Venous thrombosis**

Arterial thrombosis



Coronary arteries
=
Myocardial infarction (MI)





Brain arteries
=
Ischemic Stroke

leg arteries
=
Limb ischemia
Peripheral Artery Disease (PAD)

Arterial thrombosis : treatment

Thrombolytic therapy (fibrinolysis)

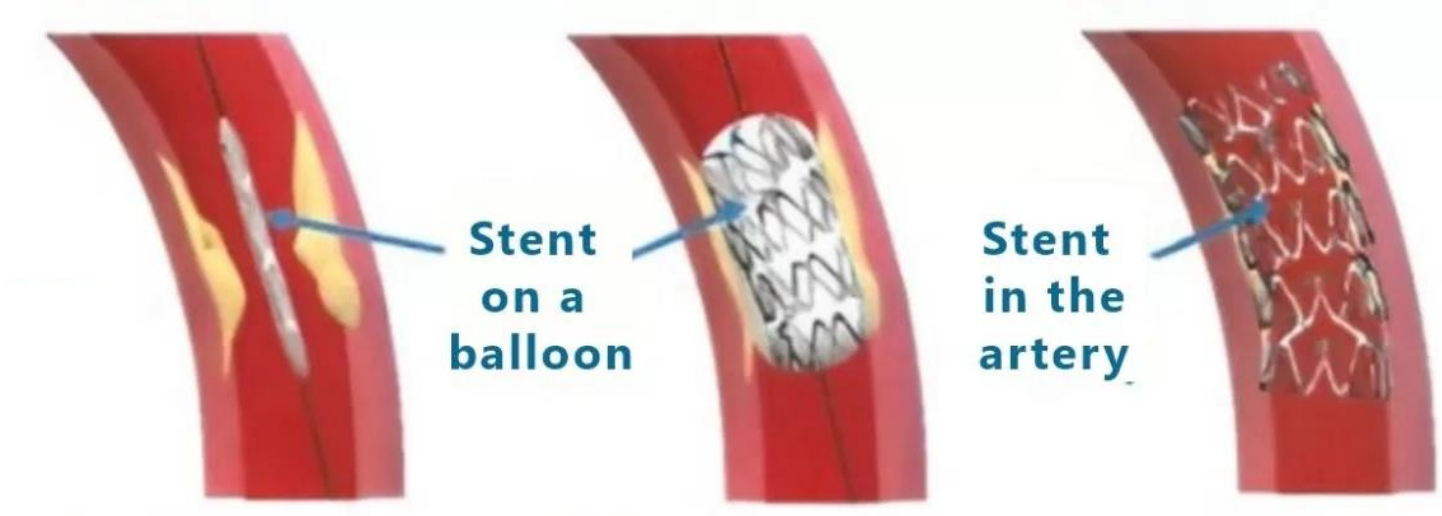
Emergency treatment for heart attacks, strokes,…

Thrombectomy

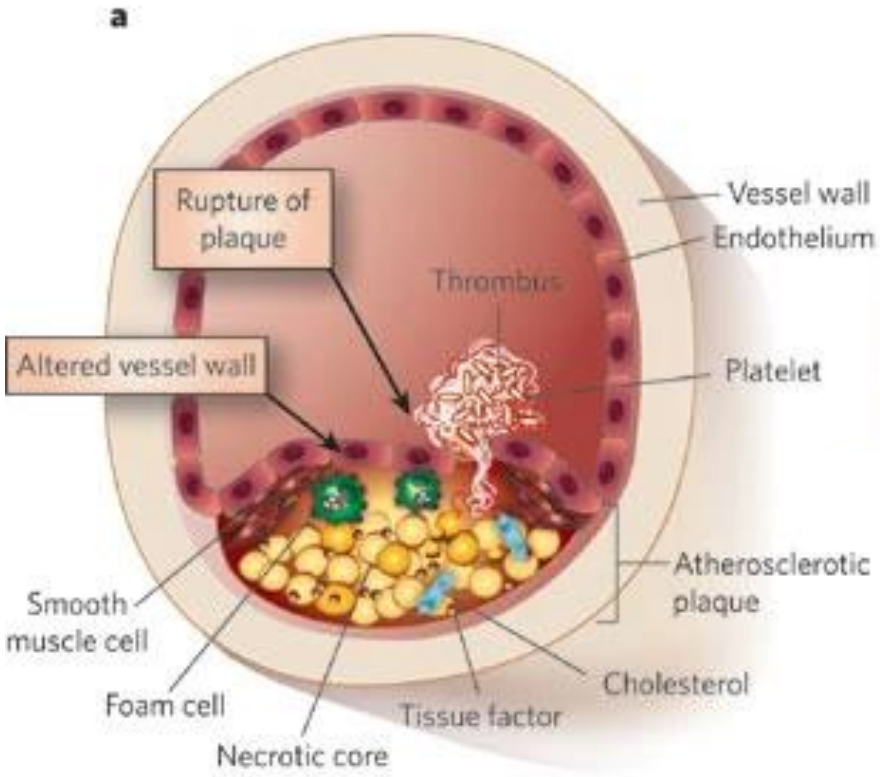
Emergency treatment for heart attacks, strokes,…

Percutaneous coronary intervention (PCI)

Emergency treatment for Myocardial infarction



Arterial thrombosis : treatment



Arterial thrombosis

Damaged vessel wall: Atherosclerosis

High shear forces

Platelet-rich thrombus

⬇

Antiplatelet drugs

Its mandatory to prevent recurrence

Atherosclerotic context

Antiplatelet drugs

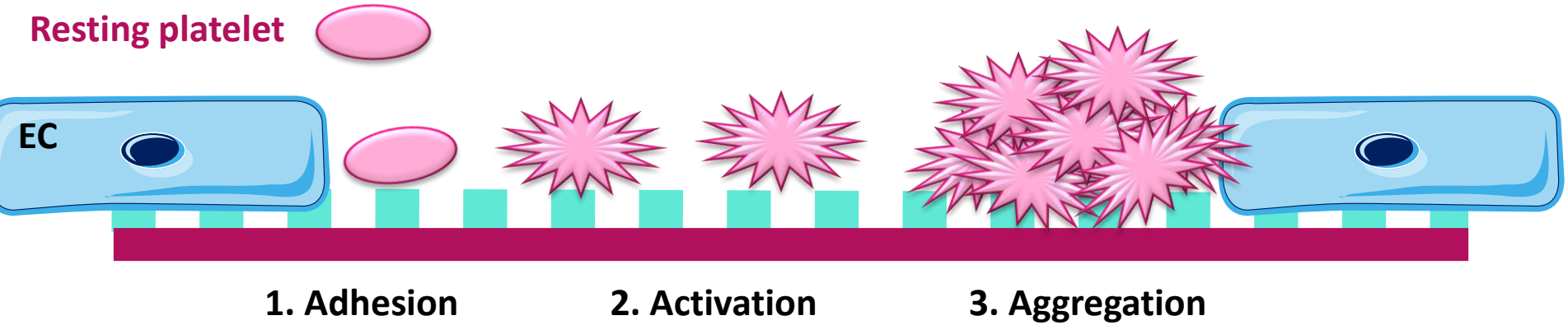
3 targets

- **COX-1**
- **P2Y12**
- **GPIIbIIIa**

Primary hemostasis

Platelet activation = 3 steps process

1. **Adhesion**
2. **Activation (cytoskeleton remodeling, secretion)**
3. **Aggregation**



Primary hemostasis

Platelet activation = 3 steps process

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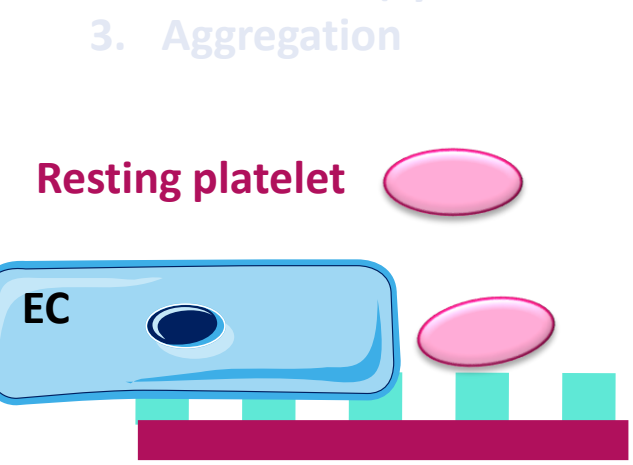
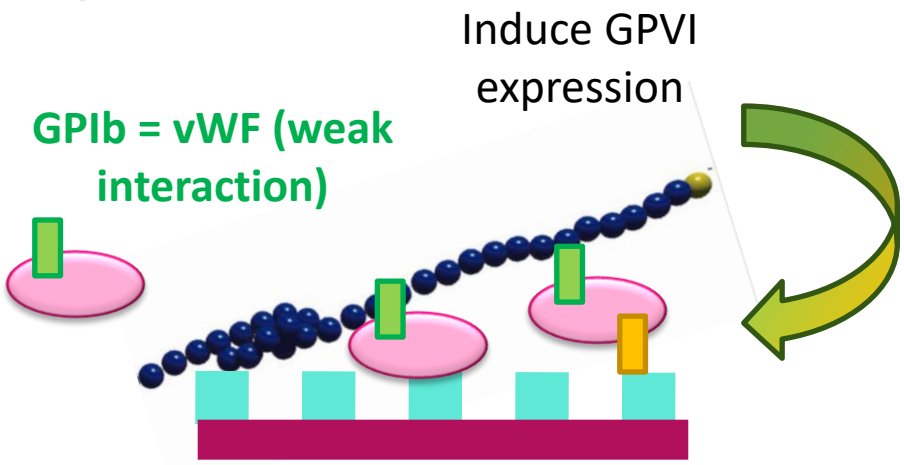
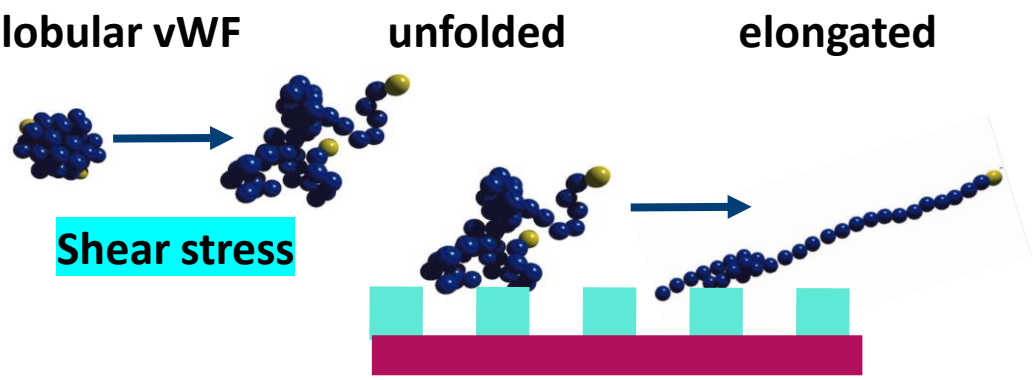




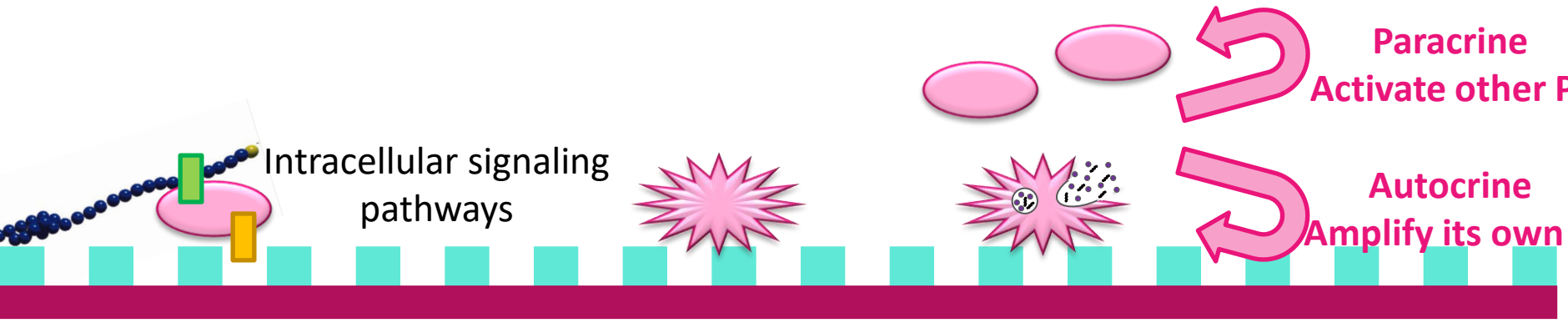
vWF secretion in plasma



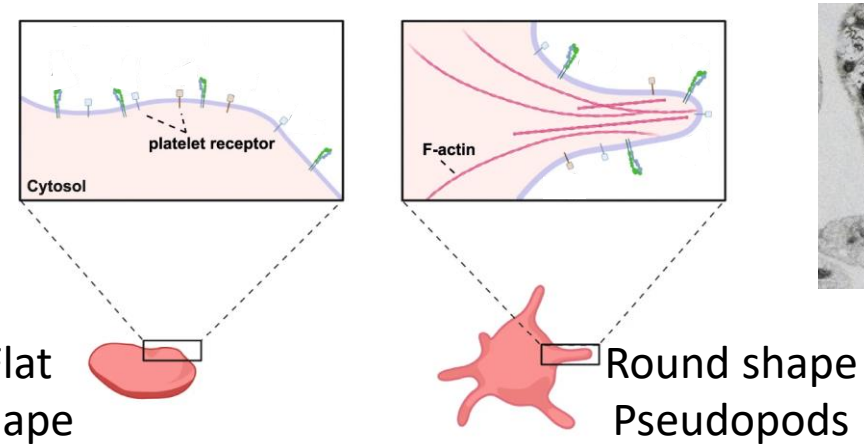
Primary hemostasis

Platelet activation = 3 steps process

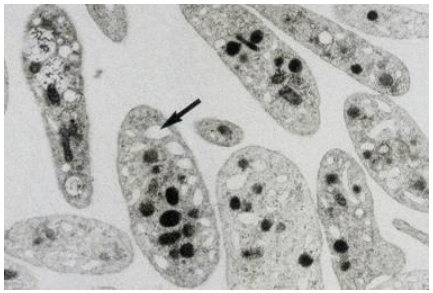
1. Adhesion
2. **Activation (cytoskeleton remodeling, secretion) = Outside-in signaling**
3. Aggregation



① Actin remodeling



② Granule secretion



α-granule

- vWF
- Fibrinogen
- PF4

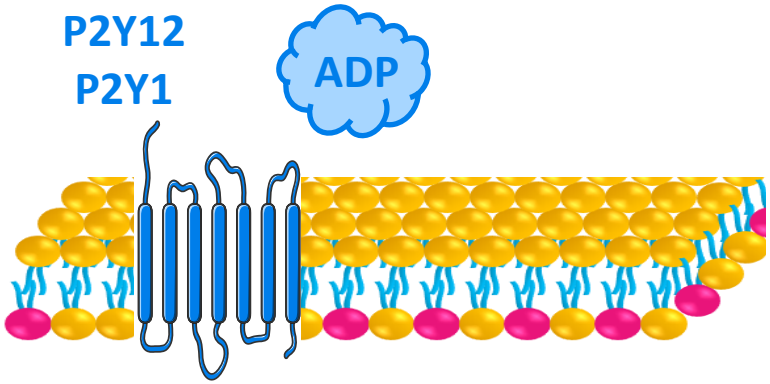
Dense granule

- **ADP**
- Serotonin
- Ca^{2+}

Primary hemostasis

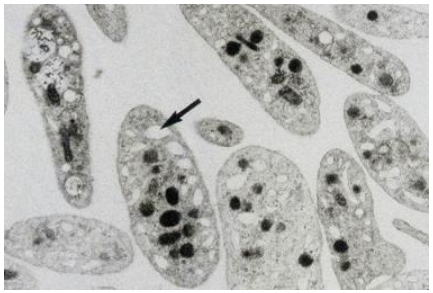
Platelet activation = 3 steps process

1. Adhesion
2. **Activation (cytoskeleton remodeling, secretion) = Outside-in signaling**
3. Aggregation



Signaling pathway

② **Granule secretion**



α-granule

- vWF
- Fibrinogen
- PF4

Dense granule

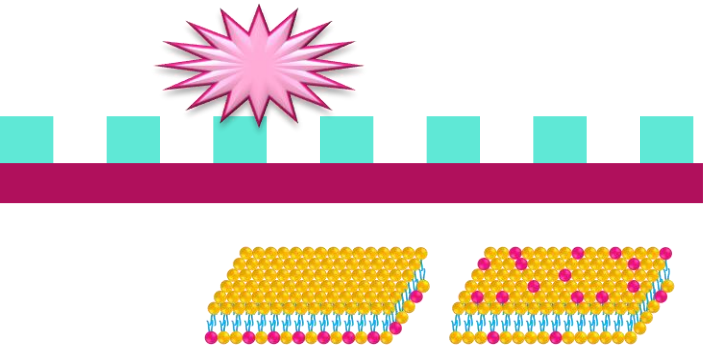
- **ADP**
- Serotonin
- Ca^{2+}

Primary hemostasis

In the same time…

Platelet activation = 3 steps process

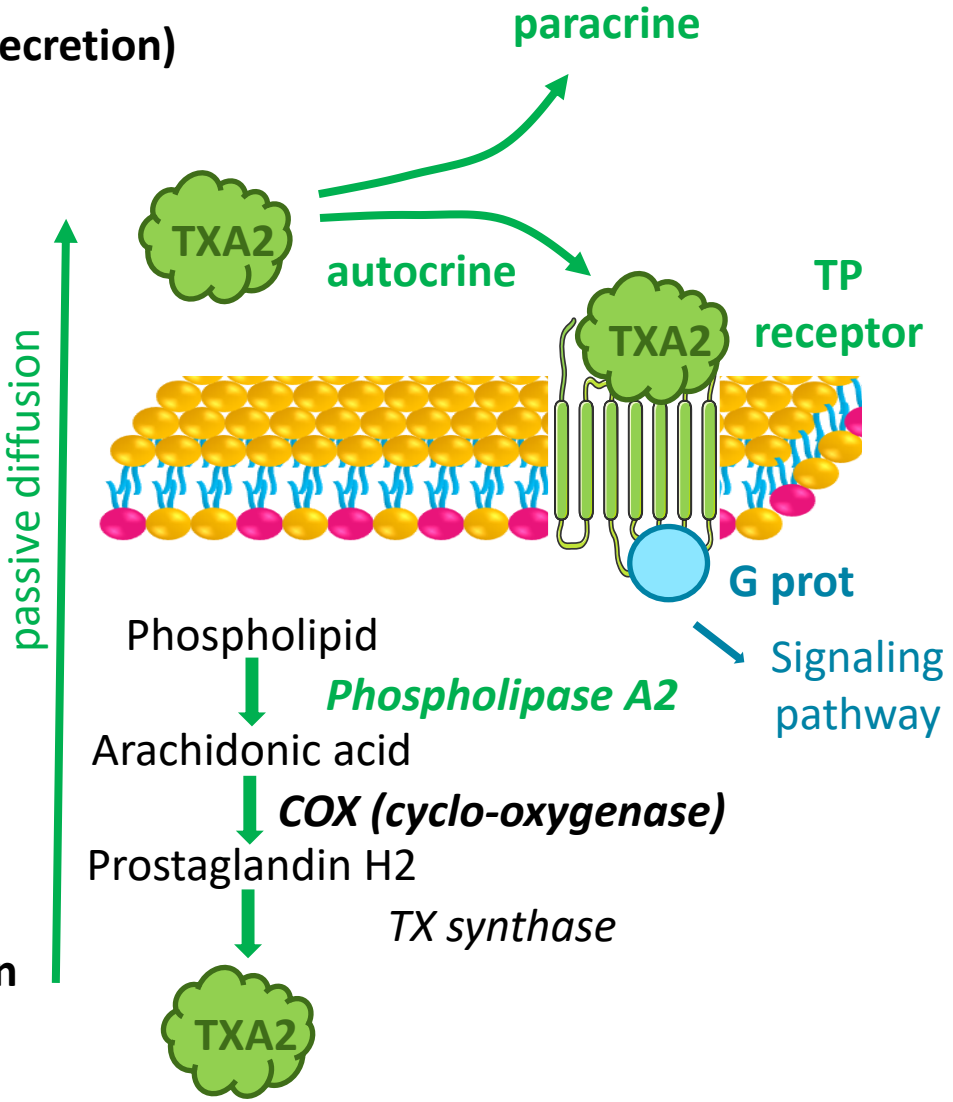
1. Adhesion
2. **Activation (cytoskeleton remodeling, secretion)**
3. Aggregation



③ Flip-Flop

- PS exposure
- Procoagulant surface

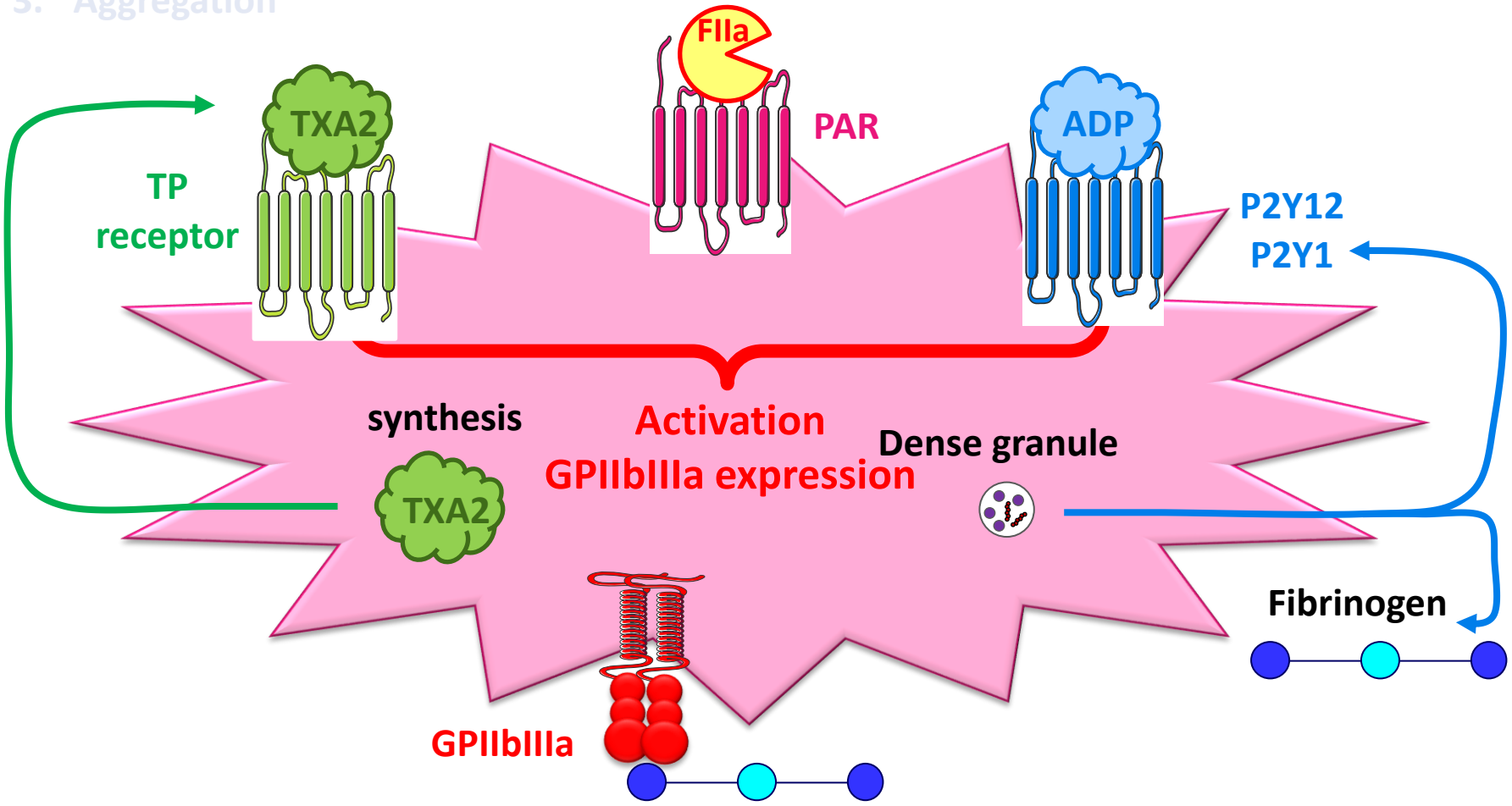
④ Thromboxane A2 (TXA2) secretion



Primary hemostasis

Platelet activation = 3 steps process

1. Adhesion
2. **Activation (cytoskeleton remodeling, secretion)**
3. Aggregation

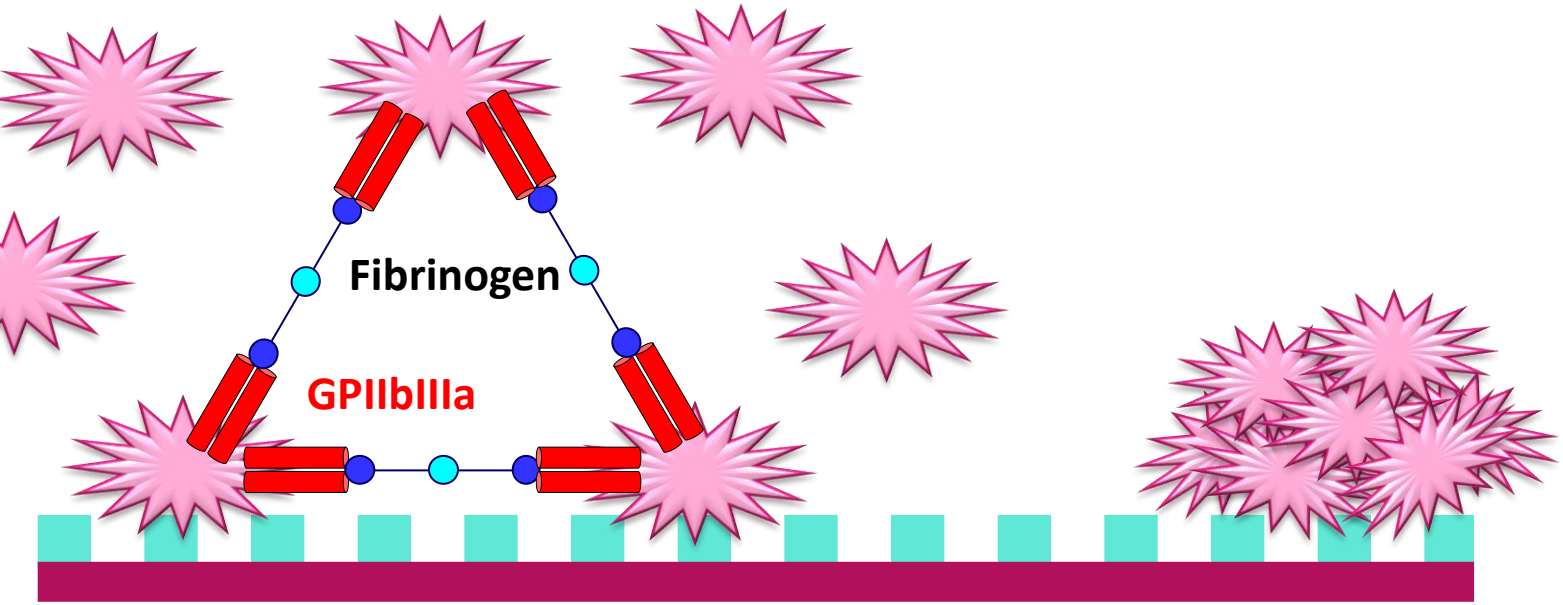


Primary hemostasis

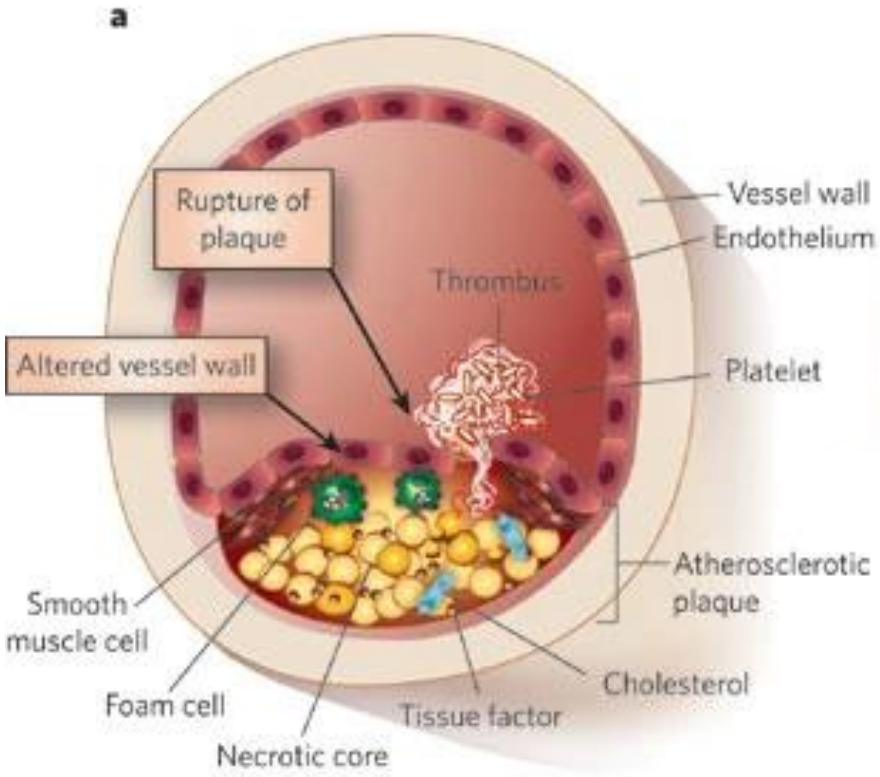
Platelet activation = 3 steps process

1. Adhesion
2. Activation (cytoskeleton remodeling, secretion)
3. **Aggregation**

GPIIbIIIa/fibrinogen complexes
bridge activated platelets



Arterial thrombosis : treatment



Arterial thrombosis

Damaged vessel wall: Atherosclerosis

High shear forces

Platelet-rich thrombus

⬇

Antiplatelet drugs

Its mandatory to prevent recurrence

Atherosclerotic context

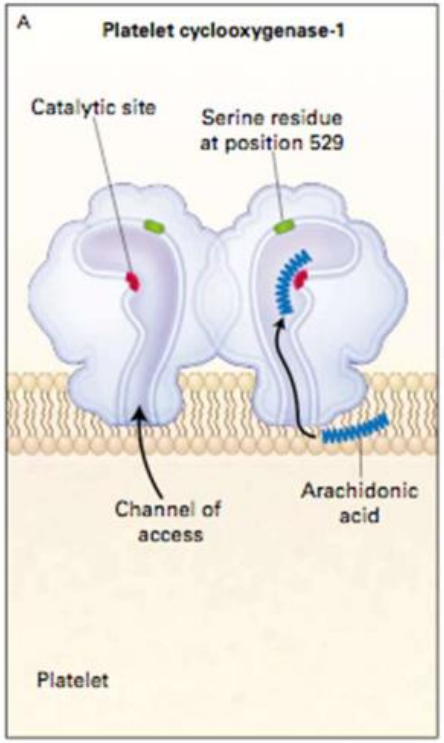
Antiplatelet drugs

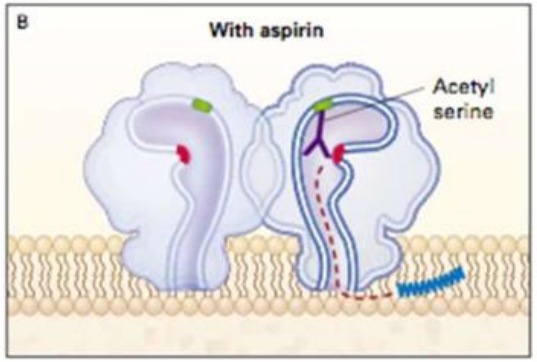
3 targets

- **COX-1 (Activation pw TXA2)**
- **P2Y12 (Activation pw ADP)**
- **GPIIbIIIa (pt aggregation)**

Antiplatelet drugs

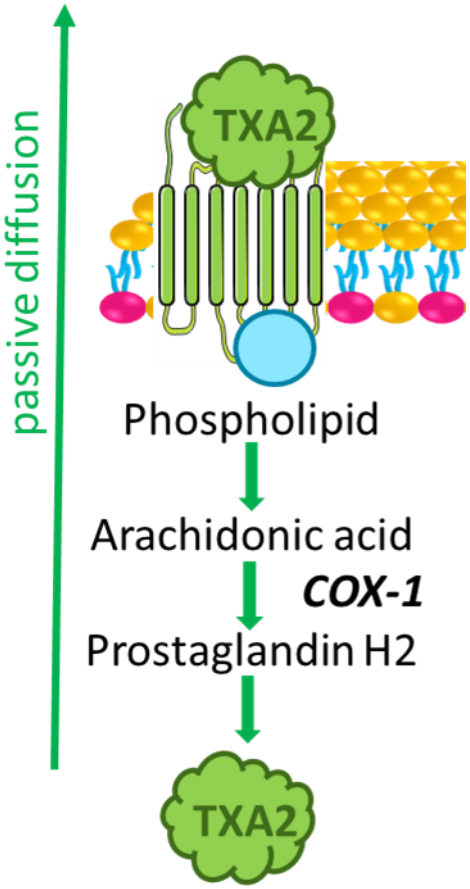
Aspirin (Acetyl-salicylic acid) = COX inhibitor





From Catella-Lawson, *N Engl J Med*, 2001

- Irreversible inhibitor of platelet COX-1
  - Persisting effect for platelet lifetime (10 days)
  - Antiplatelet effect obtained with low doses (50-100 mg/day)
- Acetylation of Serine 529
  - Away from the catalytic site



↘ TXA2 secretion ⇨

↘ platelet activation

Antiplatelet drugs

Aspirin (Acetyl-salicylic acid) = COX inhibitor

- Name and registerd in 1899 by Bayer
- Meta-analysis of more than 100 studies (antithrombotic trialists' collaboration, BMJ 2002)

  Reduces cardiovascular mortality by 15%

  Reduces nonfatal cardiovascular events by 30 %

- Gastrointestinal adverse effects

  Abdominal pain, nausea

  Ulcers, perforations ⇨ Gastrointestinal bleeding

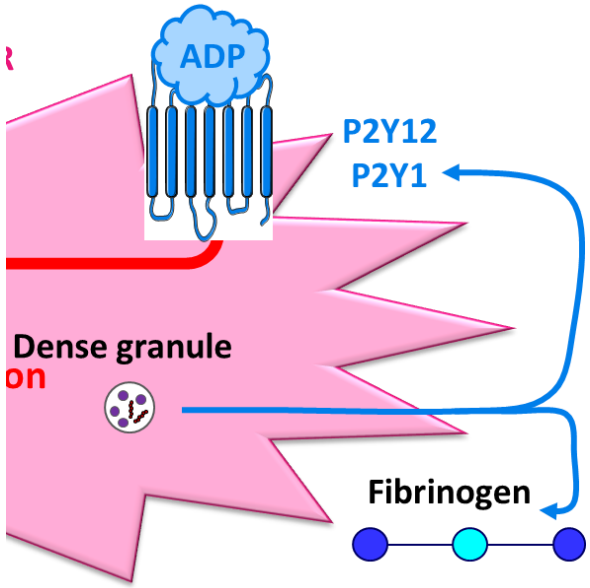
  Direct caustic effect (acidity) + antiplatelet effect (bleeding risk)

Antiplatelet drugs

P2Y12 inhibitors (ADP receptor)

Several drugs available

- ~~Ticlopidine (Ticlid®),~~ **discontinued (thrombopenia, neutropenia, anemia)**
- **Clopidogrel (Plavix®)**
- **Prasugrel (Efient®/Effient®)**
- **Ticagrelor (Brilique®, Brilinta®)**
- **Cangrelor (Kengrexal®, Kengreal®)**



Antiplatelet drugs

P2Y12 inhibitors (ADP receptor)

	Clopidogrel*	Prasugrel	Ticagrelor	Cangrelor
Approved	1997	2009	2011	2015
Receptor blockage	irreversible	irreversible	reversible	reversible
Route of adm	oral	oral	oral	IV
Prodrug	Yes	Yes	No	No
Metabolism	CYP dependent, 2 steps	CYP dependent, 1 step	Liver	No
Onset of effect	2-8 hrs	0,5-4 hrs	0,5-2 hrs	Immediate
Inhibition of platelet agreg	40–62%	70%	80–90%	>90%
Effect lasts for	7-10 days	7-10 days	3-5days	1 hr

* Numerous drug interactions and resistance, High interindividual variability

Antiplatelet drugs

P2Y12 inhibitors (ADP receptor)

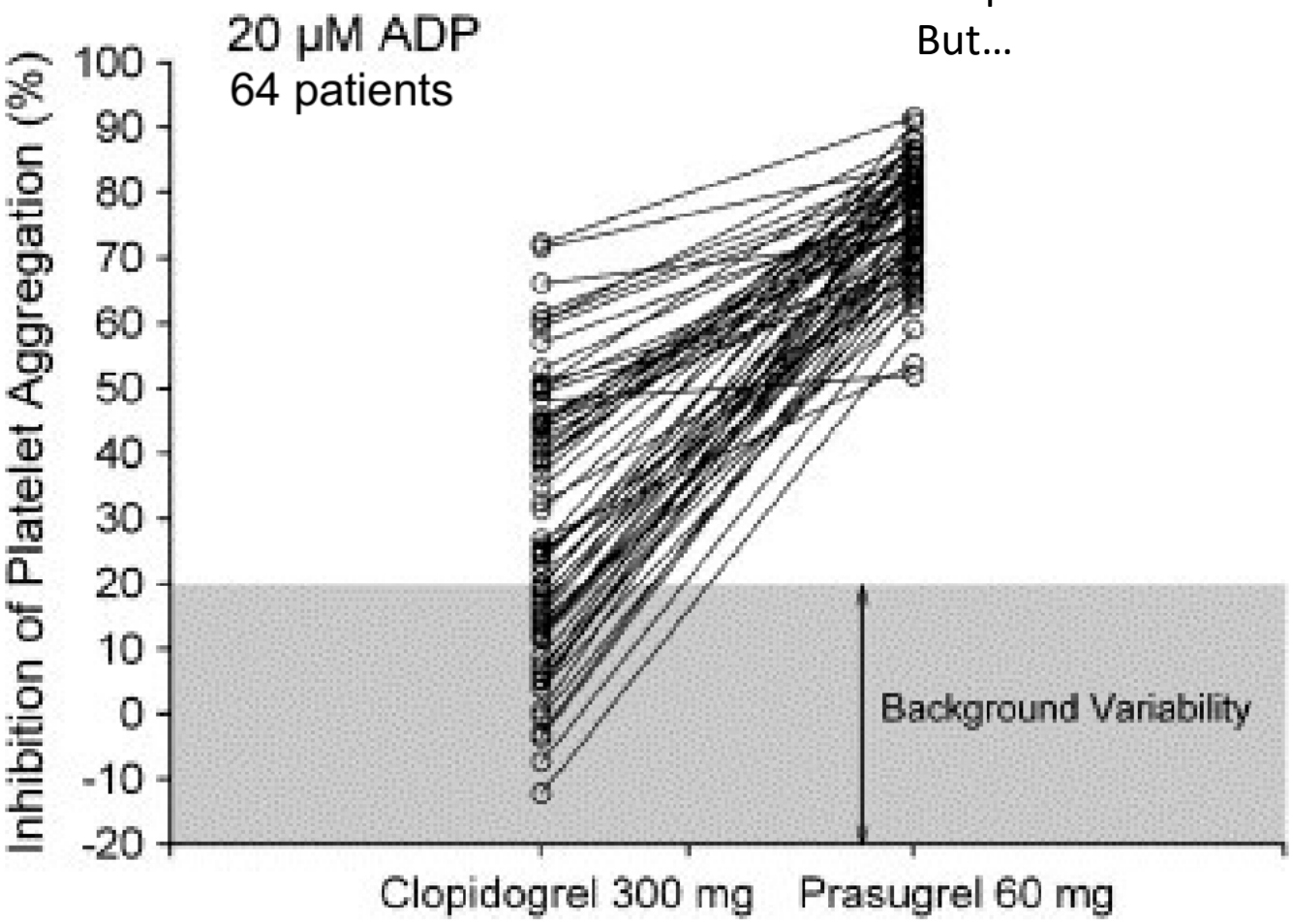
- **Less used (IV route)**
- **Not clearly superior to prasugrel and ticagrelor**
- **Approved for patients undergoing PCI, transition to an oral P2Y12 inhibitor after procedure**

	Cangrelor
Approved	2015
Receptor blockage	reversible
Route of adm	IV
Prodrug	No
Metabolism	No
Onset of effect	Immediate
Inhibition of platelet agreg	>90%
Effect lasts for	1 hr

Antiplatelet drugs

P2Y12 inhibitors (ADP receptor)

Prasugrel ↘ interindividual variability
Simpler metabolism
But…

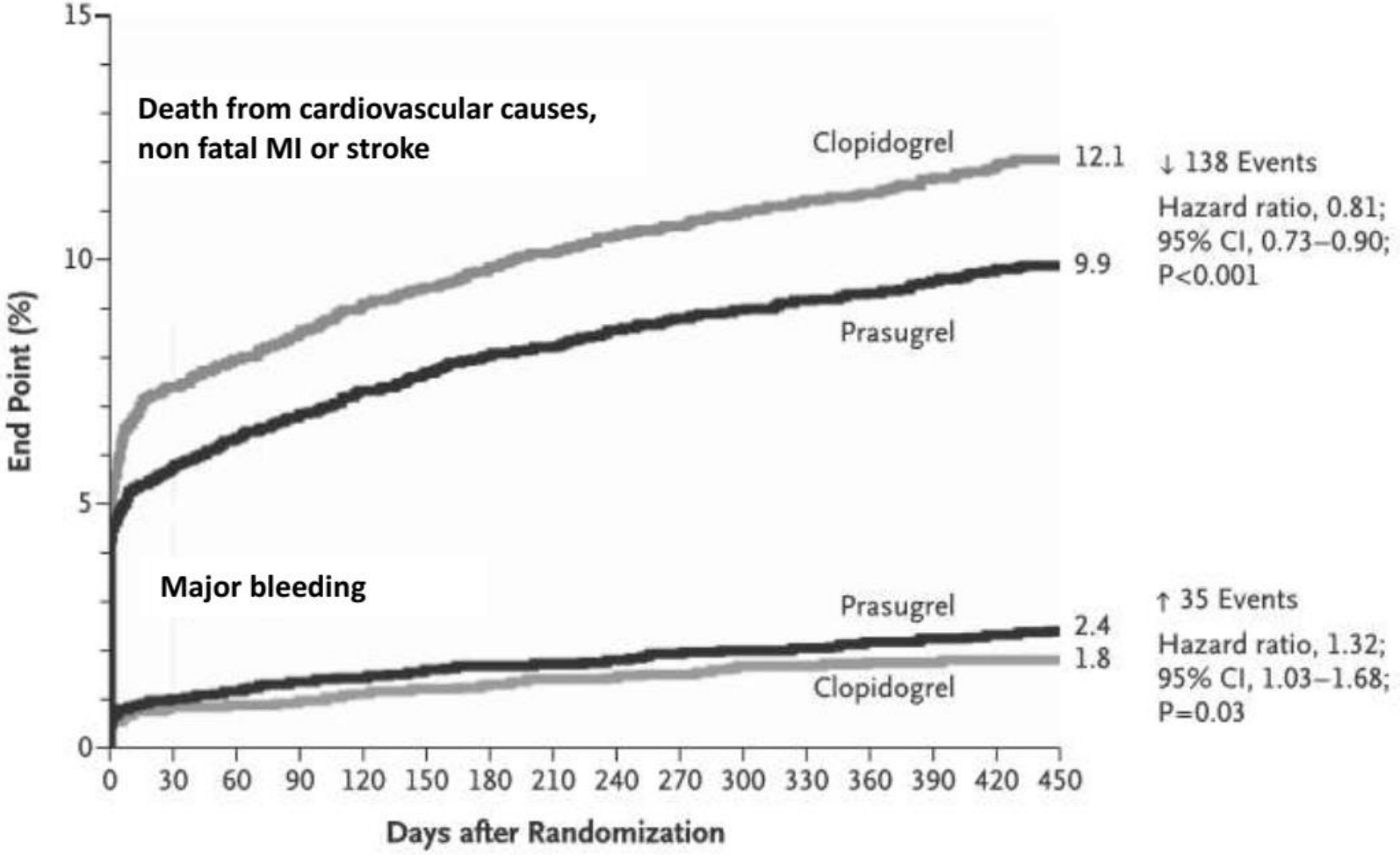


Brandt JT et al, *Am Heart J*, 2007

Antiplatelet drugs

P2Y12 inhibitors (ADP receptor)

Prasugrel more powerful, but slightly less safe than clopidogrel

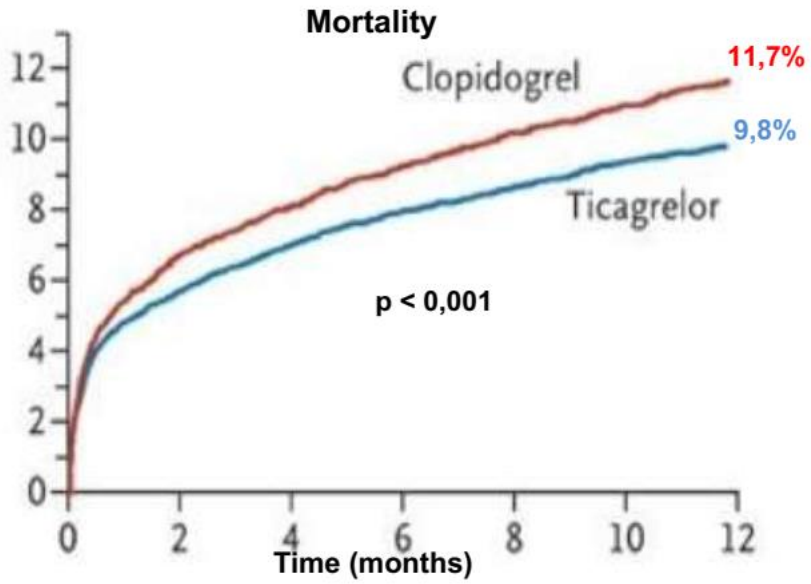


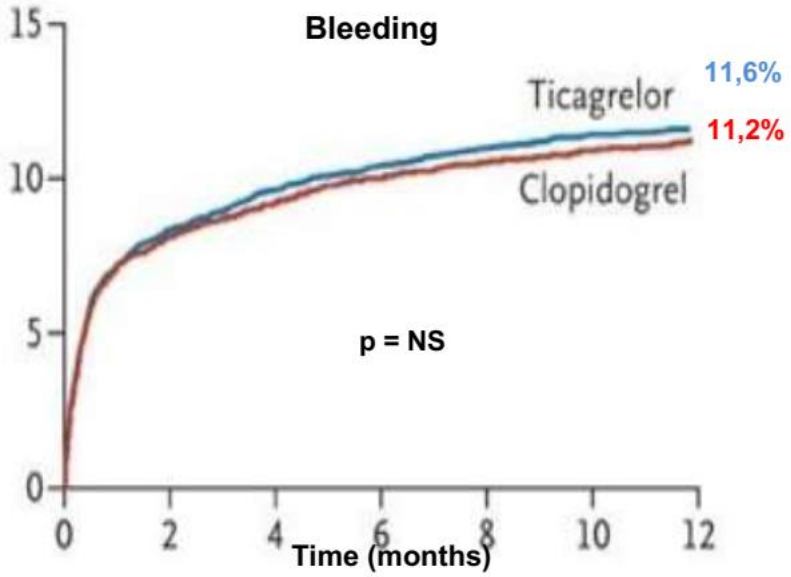
From Wiviott et al, *N Engl J Med*, 2007

Antiplatelet drugs

P2Y12 inhibitors (ADP receptor)

Ticagrelor more powerful,
and as safe as clopidogrel
But…

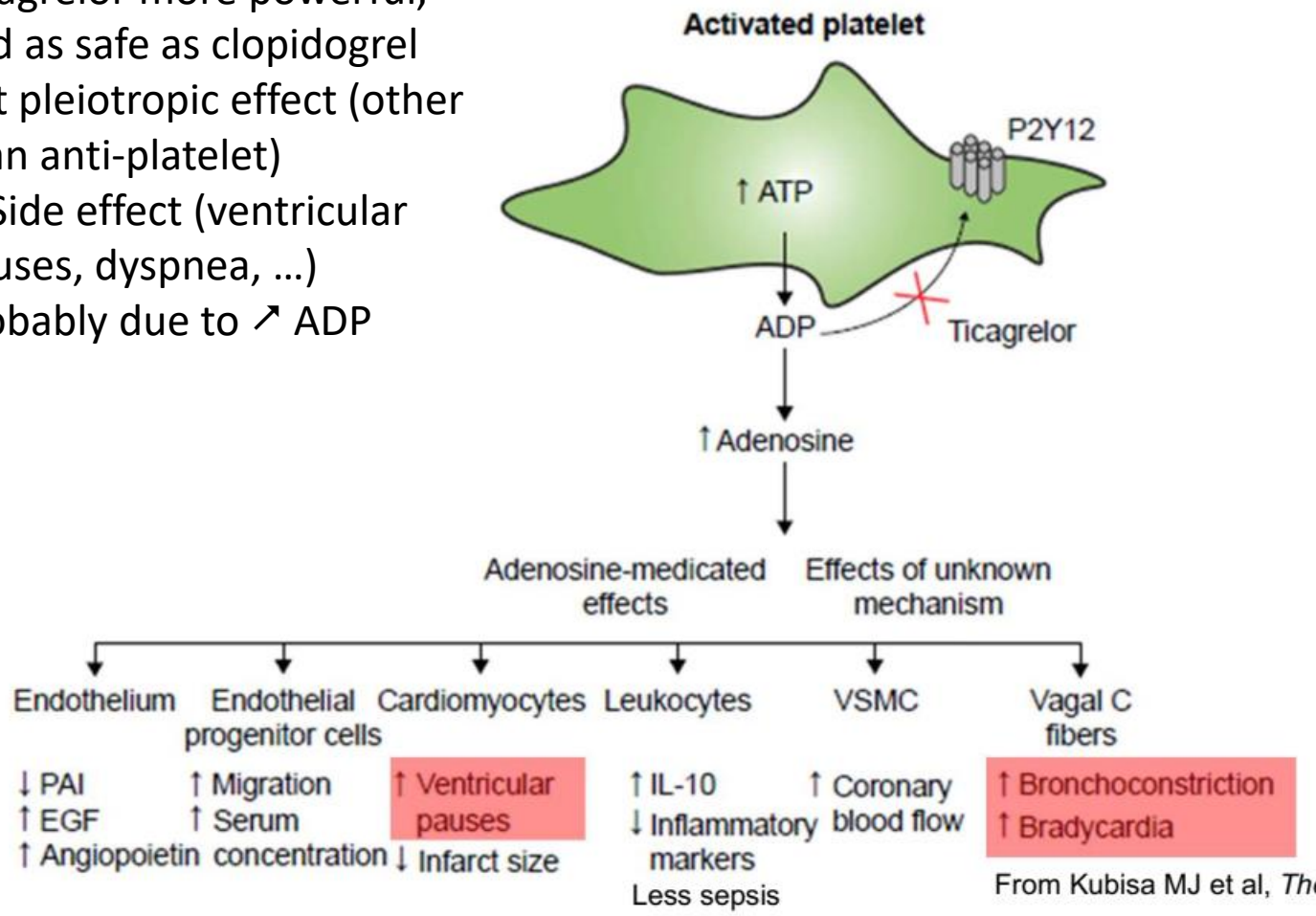




Antiplatelet drugs

P2Y12 inhibitors (ADP receptor)

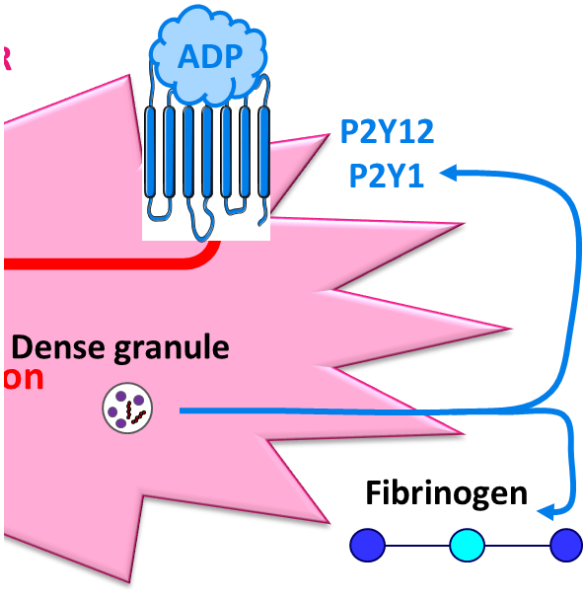
Ticagrelor more powerful, and as safe as clopidogrel
But pleiotropic effect (other than anti-platelet)
↗ Side effect (ventricular pauses, dyspnea, …)
Probably due to ↗ ADP

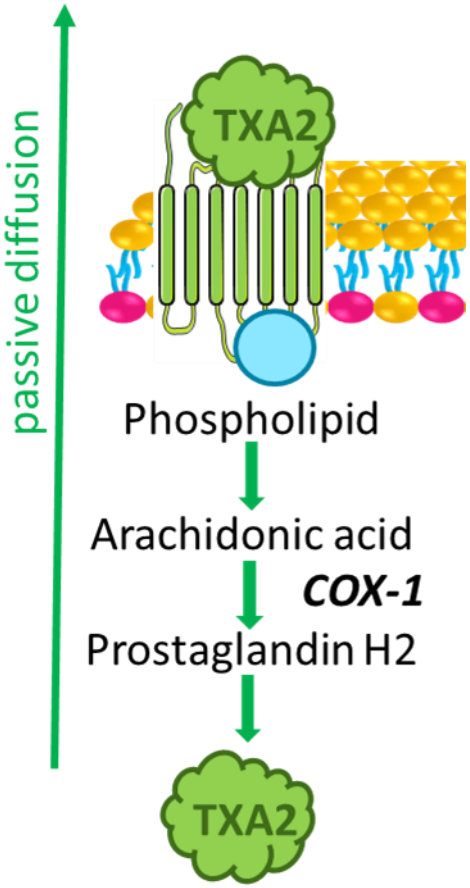


From Kubisa MJ et al, *Ther Clin Risk Manag*, 2018

Antiplatelet drugs

Dual therapy : P2Y12 inhibitors + Aspirin

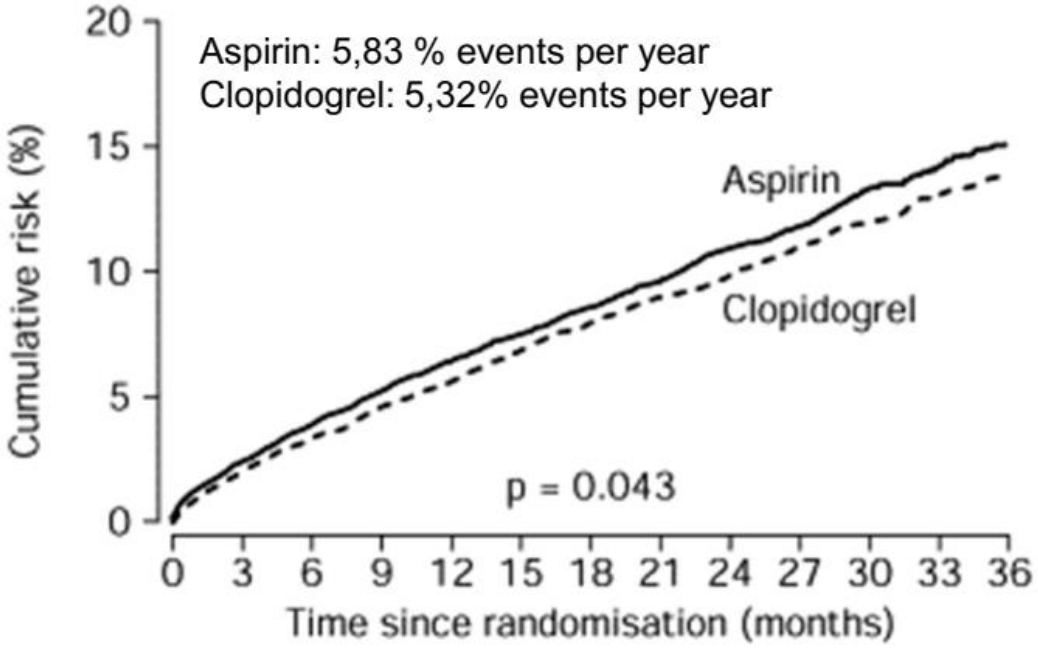




Different mechanisms of action (one doesn't replace the other) ⇨ Dual therapy to increase the antiplatelet effect through inhibition of 2 distinct pathways

Antiplatelet drugs

Dual therapy : P2Y12 inhibitors + Aspirin



From CAPRIE Steering Committee, *Lancet*, 1996

CAPRIE study

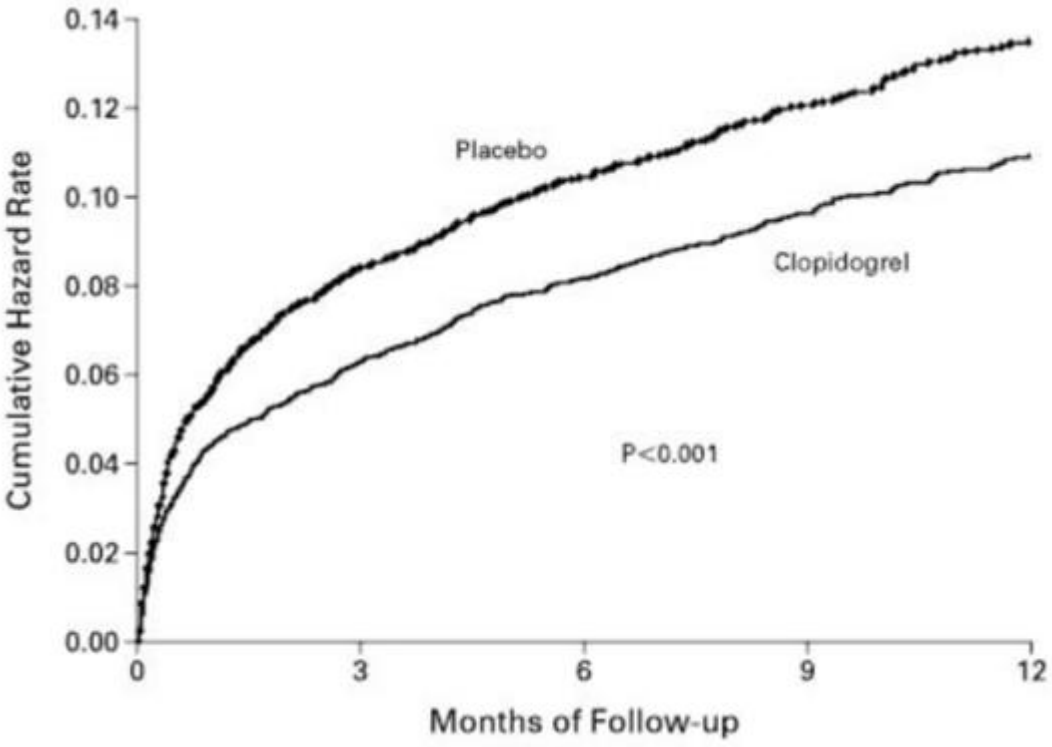
- ~20 000 patients with recent MI, stroke, or PAD
- Treated with clopidogrel or aspirin
- Assessment of major cardiovascular complications under therapy

Both have similar efficacy

Antiplatelet drugs

Ex clopidogrel + Aspirin (Duoplavin®)

Dual therapy : P2Y12 inhibitors + Aspirin



CURE study

- ~10 000 patients with recent arterial thrombosis diagnosis
- Treated with aspirin + placebo or aspirin + clopidogrel
- Assessment of major cardiovascular complications

Dual therapy has higher efficacy

But higher bleeding risk

From Yusuf S et al, *N Engl J Med*, 2001

	Asp + placebo	Asp + Clopidogrel
Cardiovascular events	11,4 %	9,3% (p<0,001)
Major bleedings	2,7 %	3,7 % (p<0,001)

Antiplatelet drugs

Block platelets aggregation (~~activation~~)

GPIIbIIIa inhibitors (fibrinogen binding)

Abciximab (REOPRO®):

– monoclonal anti-GPIIbIIIa antibody (hinders RGD binding site)

– Irreversible

Eptifibatide (INTEGRILIN®)

– Peptide derived from rattlesnake venom

– KGD sequence (binds to RGD binding site, competes with fibrinogen)

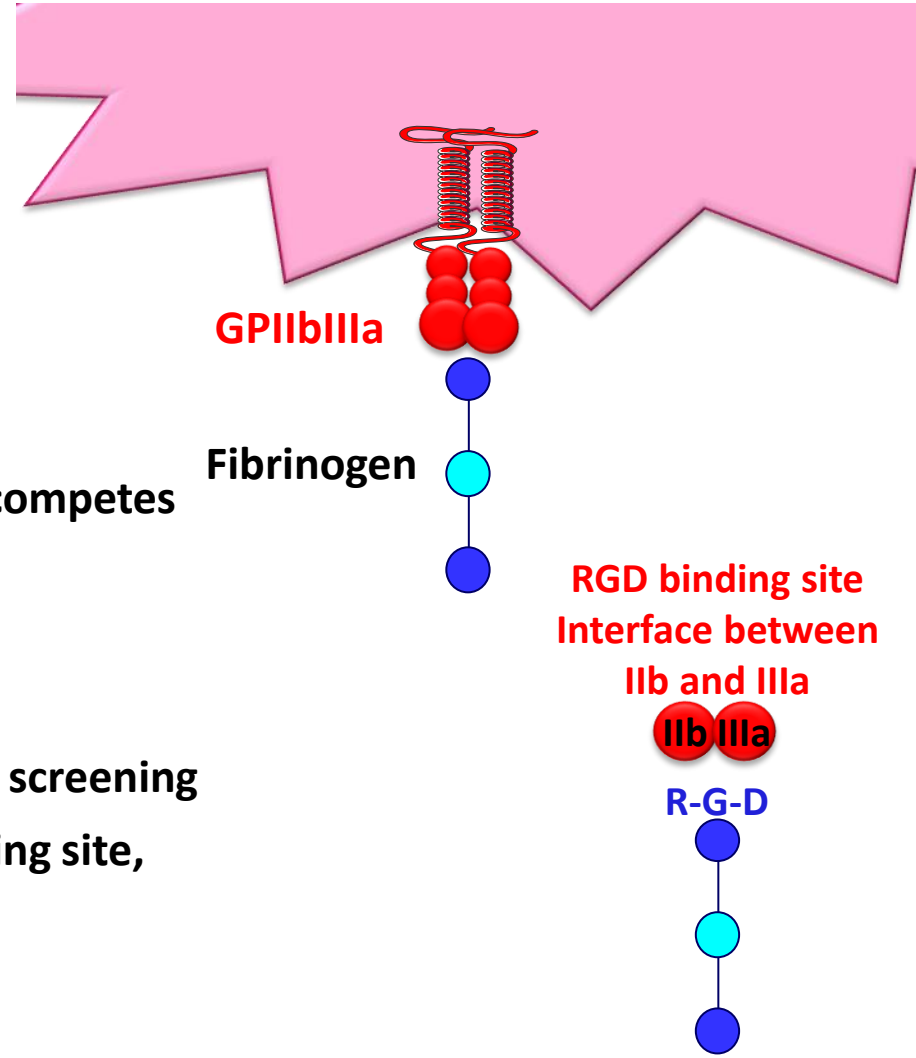
– Reversible

Tirofiban (AGGRASTAT®)

– Non peptidic molecule identified by virtual screening

– Structure mimicks RGD (binds to RGD binding site, competes with fibrinogen)

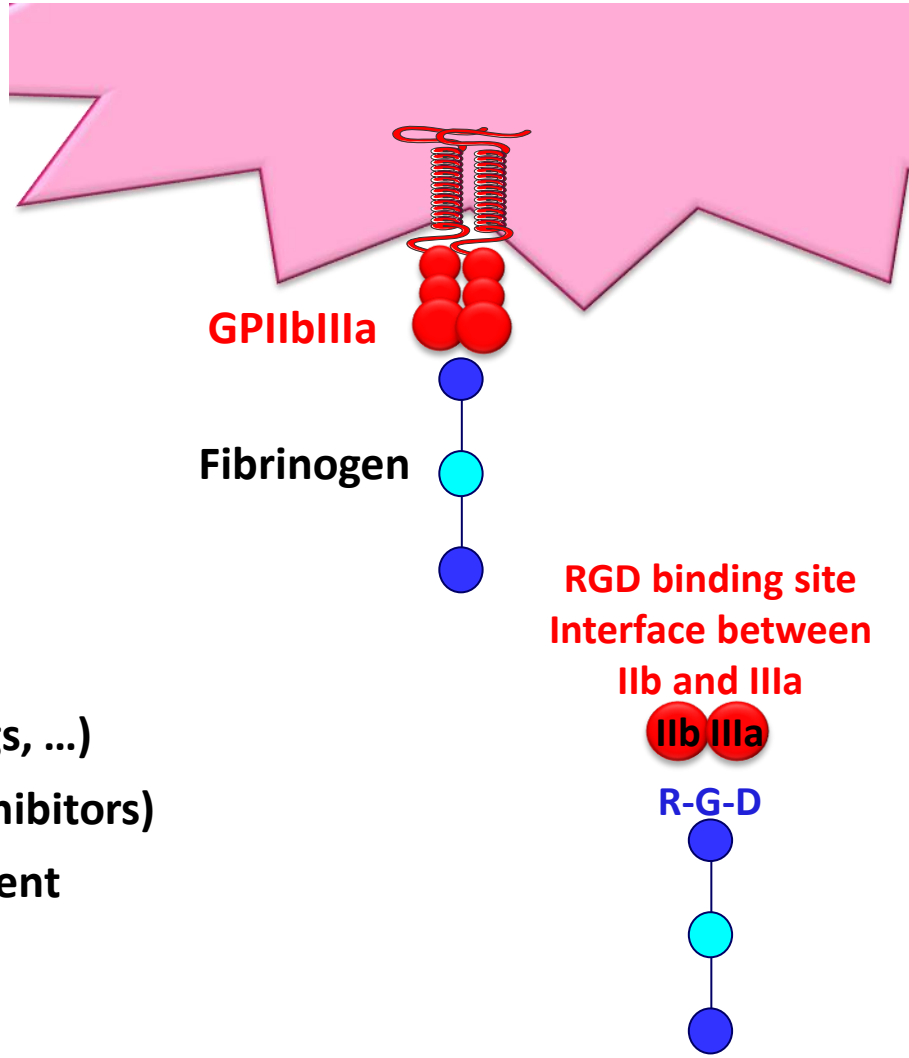
– Reversible



Antiplatelet drugs

Block platelets aggregation (~~activation~~)

GPIIbIIIa inhibitors (fibrinogen binding)



IV route only

Adverse effects (thrombocytopenia, bleedings, …)

Decreasing use (mostly replaced by P2Y12 inhibitors)

Salvage situations in case of massive MI or stent thrombosis during PCI procedure)

Antiplatelet drugs

Indication summary

Indication	Drug
Stable coronary artery diseases	Long term aspirin (clopidogrel in case of allergy)
PCI (following MI)	Aspirin + Prasugrel or Aspirin + Ticagrelor for 1 year and then aspirin long term
PCI in stable coronary artery disease	Aspirin + Clopidogrel for 6-12 months and then aspirin long term
After ischemic stroke	Long term aspirin (clopidogrel in case of allergy)
PAD	Long term aspirin (clopidogrel in case of allergy)