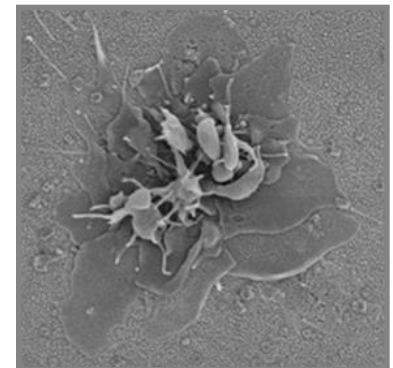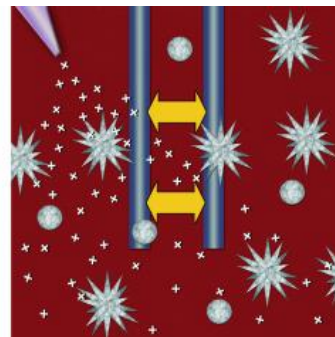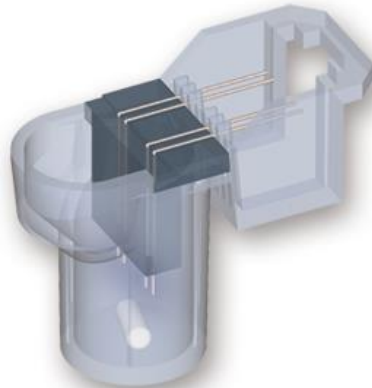# Individualization of Antiplatelet Therapy using Multiplate Analysis

Andreas Calatzis
Institute for Prevention of Cardiovascular Diseases
University of Munich

Platelet Function
Main Mechanisms
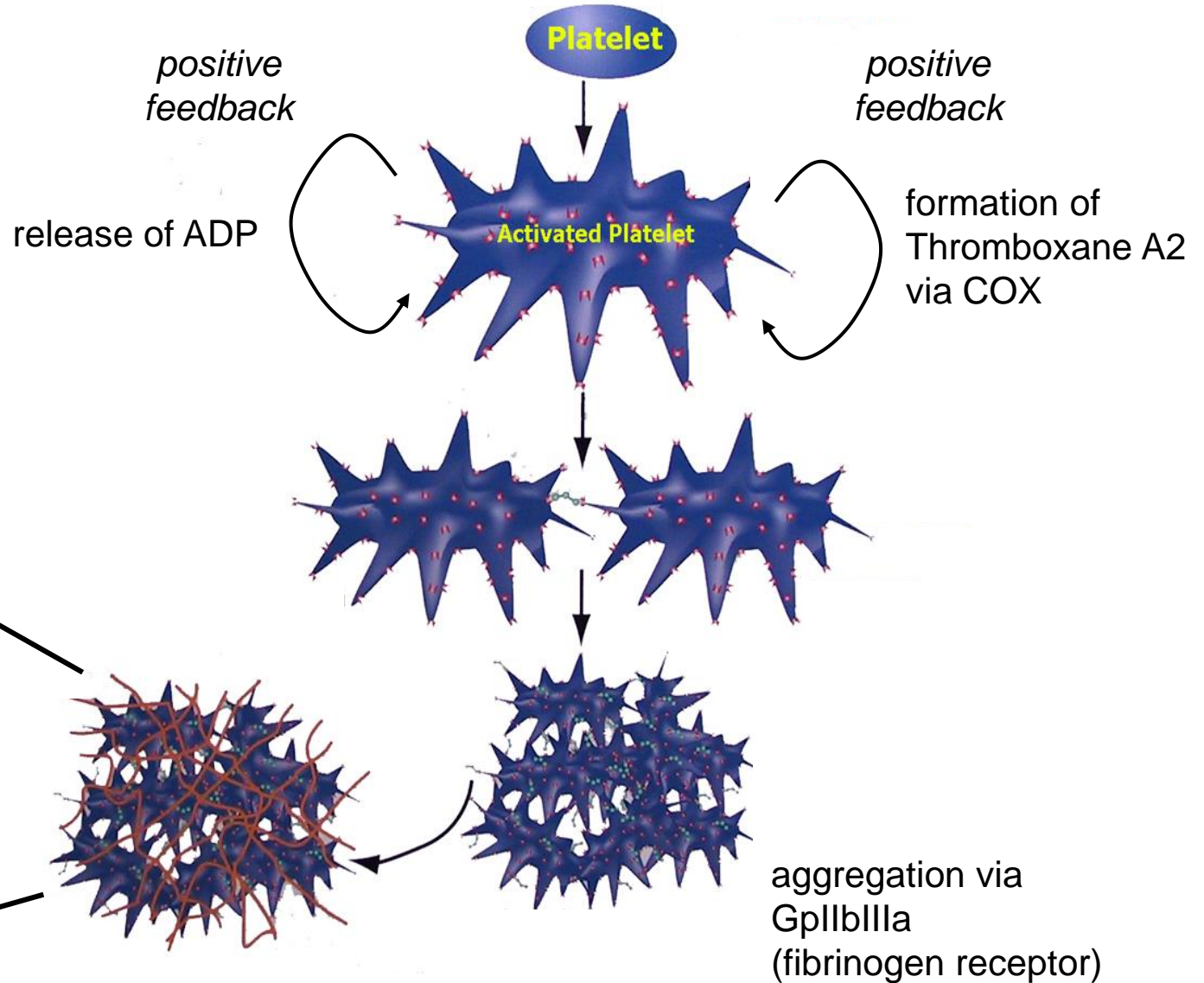
positive feedback

release of ADP

positive feedback

formation of Thromboxane A2 via COX

Platelet

Activated Platelet

hemostasis during operation, trauma, menstruation, after injuries

stent thrombosis
myocardial infarction
ischemic stroke
other ischemic events

aggregation via GpIIbIIIa
(fibrinogen receptor)

ADP Receptor Antagonists and Aspirin
Main Mechanisms

Efient prasugrel tablets

BRILIQUE 90 mg ticagrelor

Platelet

positive feedback

release of ADP

Activated Platelet

formation of Thromboxane A2 via COX

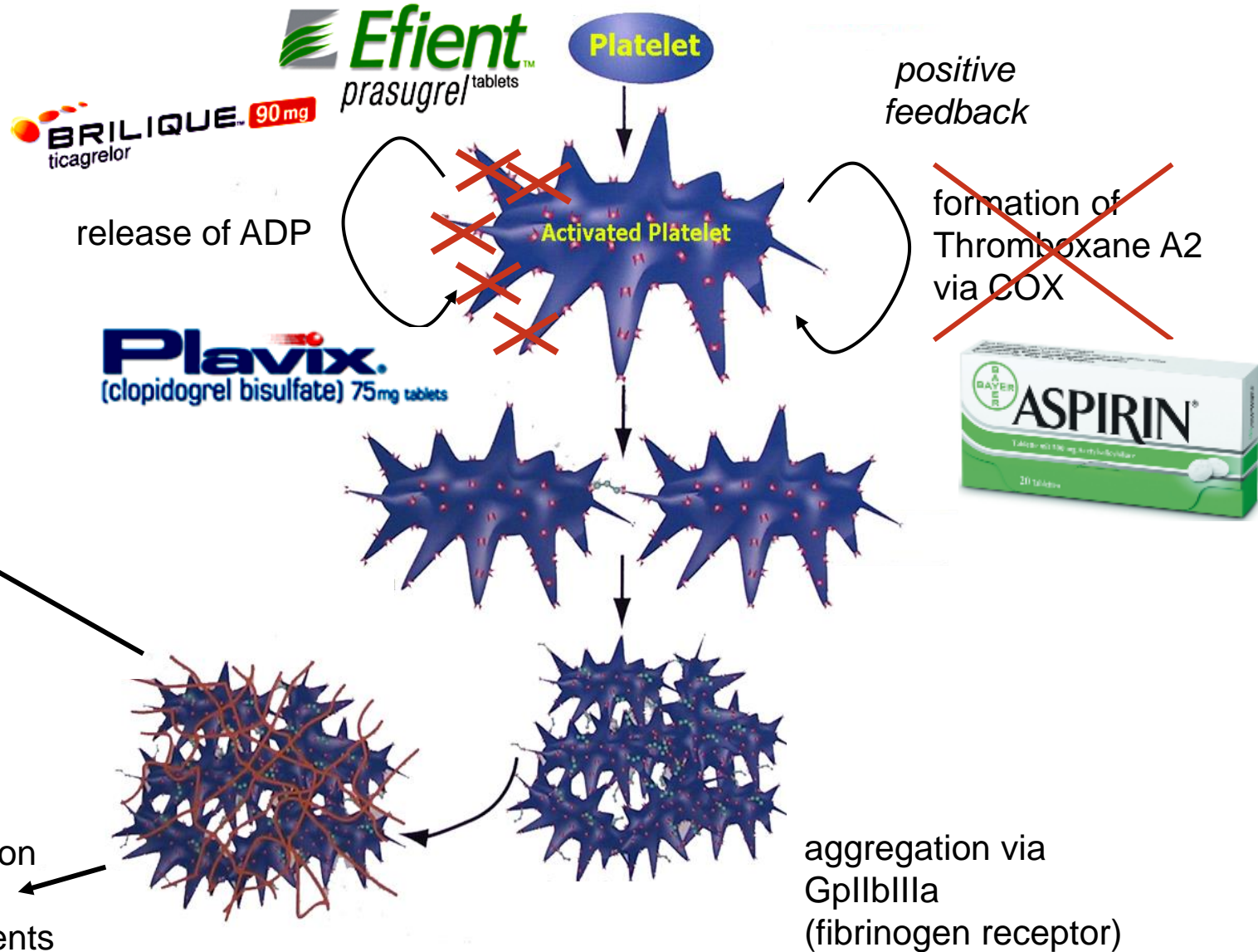**Plavix** (clopidogrel bisulfate) 75 mg tablets

ASPIRIN BAYER

hemostasis during operation, trauma, menstruation, after injuries

stent thrombosis
myocardial infarction
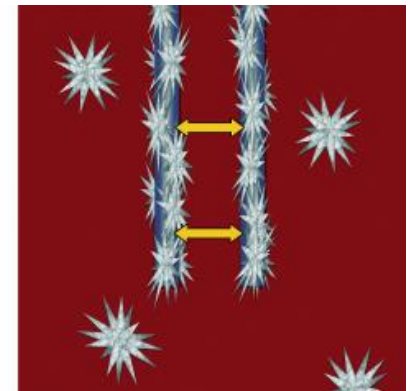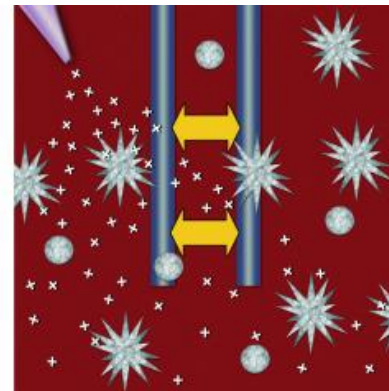ischemic stroke
other ischemic events

aggregation via GpIIbIIIa (fibrinogen receptor)
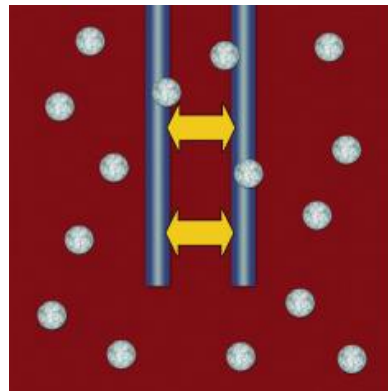
Multiplate®
Detection principle
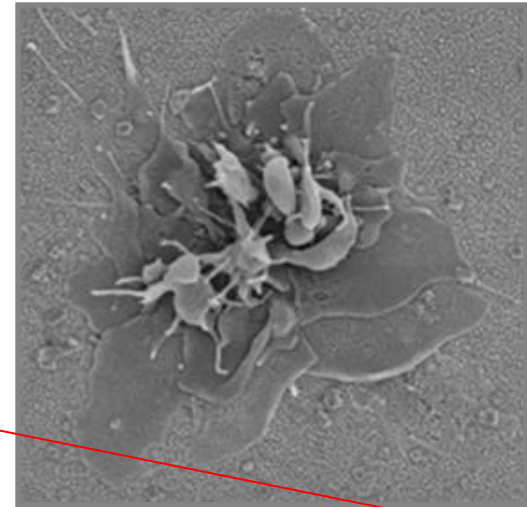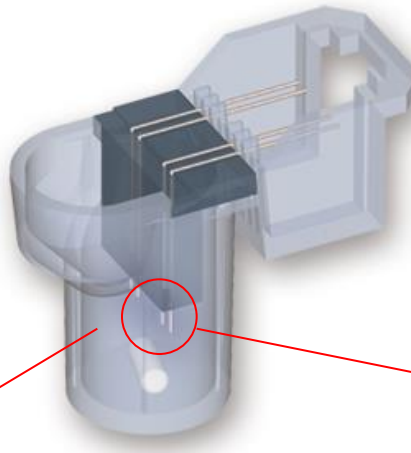
- analysis of platelet function in whole blood

- twin impedance sensor

- platelets aggregate on metal sensors and increase electrical resistance

- platelet function analysis on surfaces

Multiplate®
analyzer

- 5 channels for parallel tests

- electronic pipetting

- applicable for laboratory and near patient analysis

Multiplate Analyzer
> 200 medline-listed publications
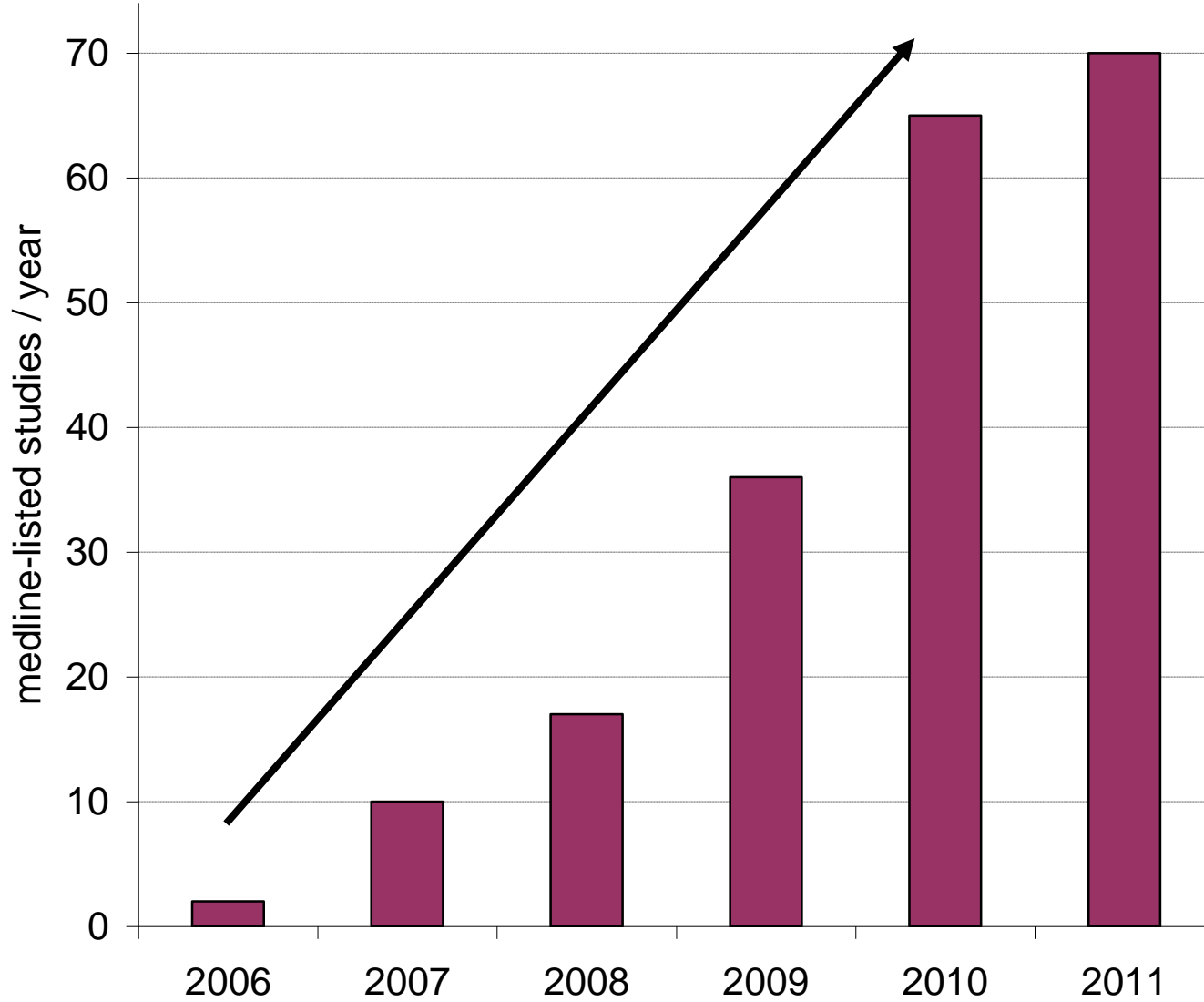
Multiplate Analyzer
Publications in Excellent Journals

Impact factor

6x	JACC	*12.6*
9x	journal of thrombosis and haemostasis	*6.1*
22x	Thrombosis and Haemostasis — International Journal for Vascular Biology and Medicine	*4.5*
3x	AHJ — American Heart Journal	*4.4*
10x	ANESTHESIA & ANALGESIA	*3.1*
15x	platelets	*2.3*

Multiplate
Parameters
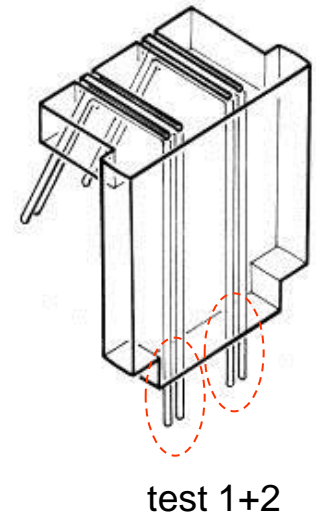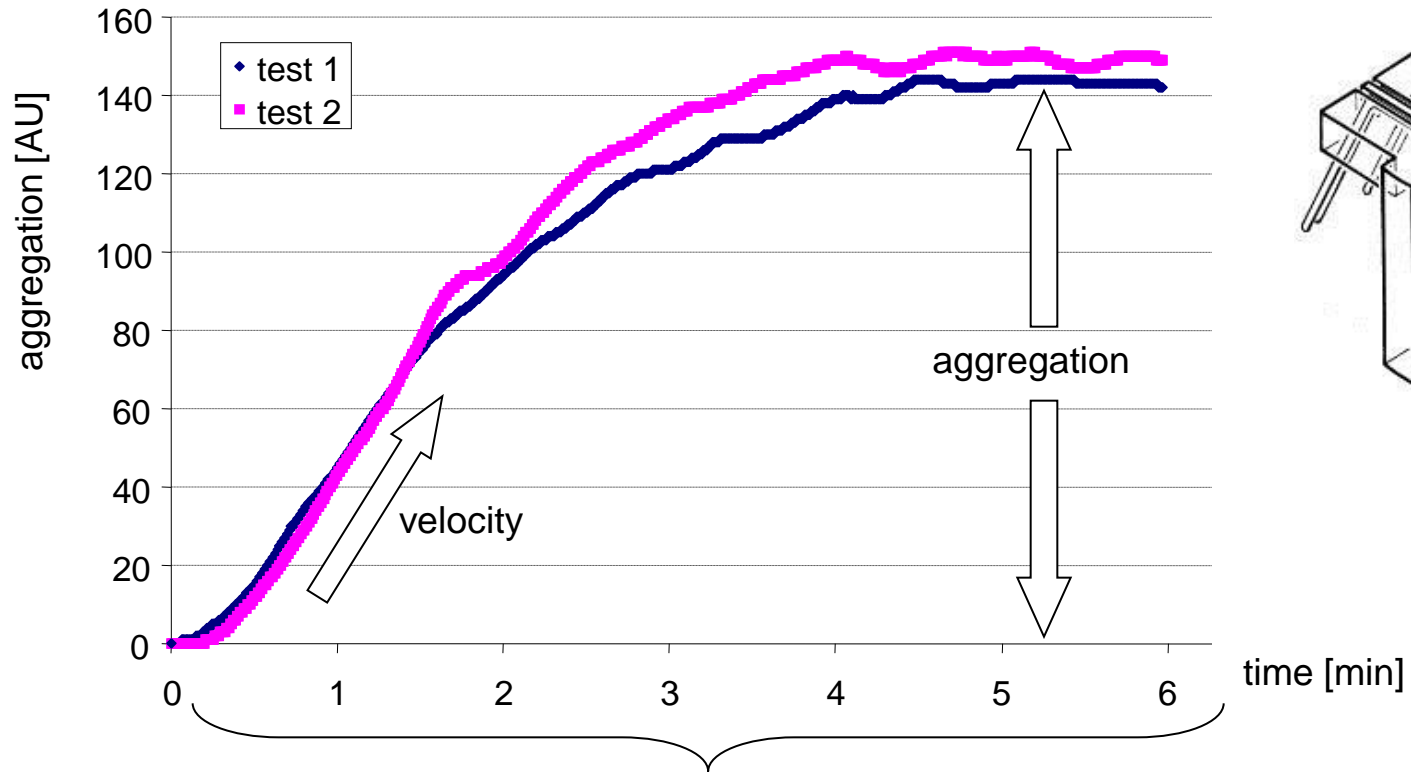
- *most important parameter*
- *expressed in AU*min or U (10 AU*min = 1 U)*

Multiplate
Main tests

Thrombin Receptor Antagonists

TRAPtest

TRAP

ADPtest

Clopidogrel
Prasugrel
Ticagrelor

ADP

TXA$_2$

TXA$_2$

COX

ASPItest

Arachidonic
Acid

Aspirin ®
NSAID

GpIIb/IIIa antagonists:

Reopro ® (abciximab)
Aggrastat ® (Tirofiban)
Integrillin ® (Eptifibatid)

resting platelet

platelet activation
GpIIb/IIIa receptor
exposure
degranulation

activated platelet

→ activation
--→ inhibition

Multiplate tracings
Examples

	TRAPtest	ASPItest	ADPtest
no platelet inhibition	113 U	102 U	89 U
100 mg aspirin qd	139 U	17 U	134 U
75 mg clopidogrel qd	98 U	89 U	31 U
100 mg aspirin + 75 mg clopidogrel qd	88 U	8 U	17 U
tirofiban (Aggrastat® i.v.)	7 U	3 U	3 U
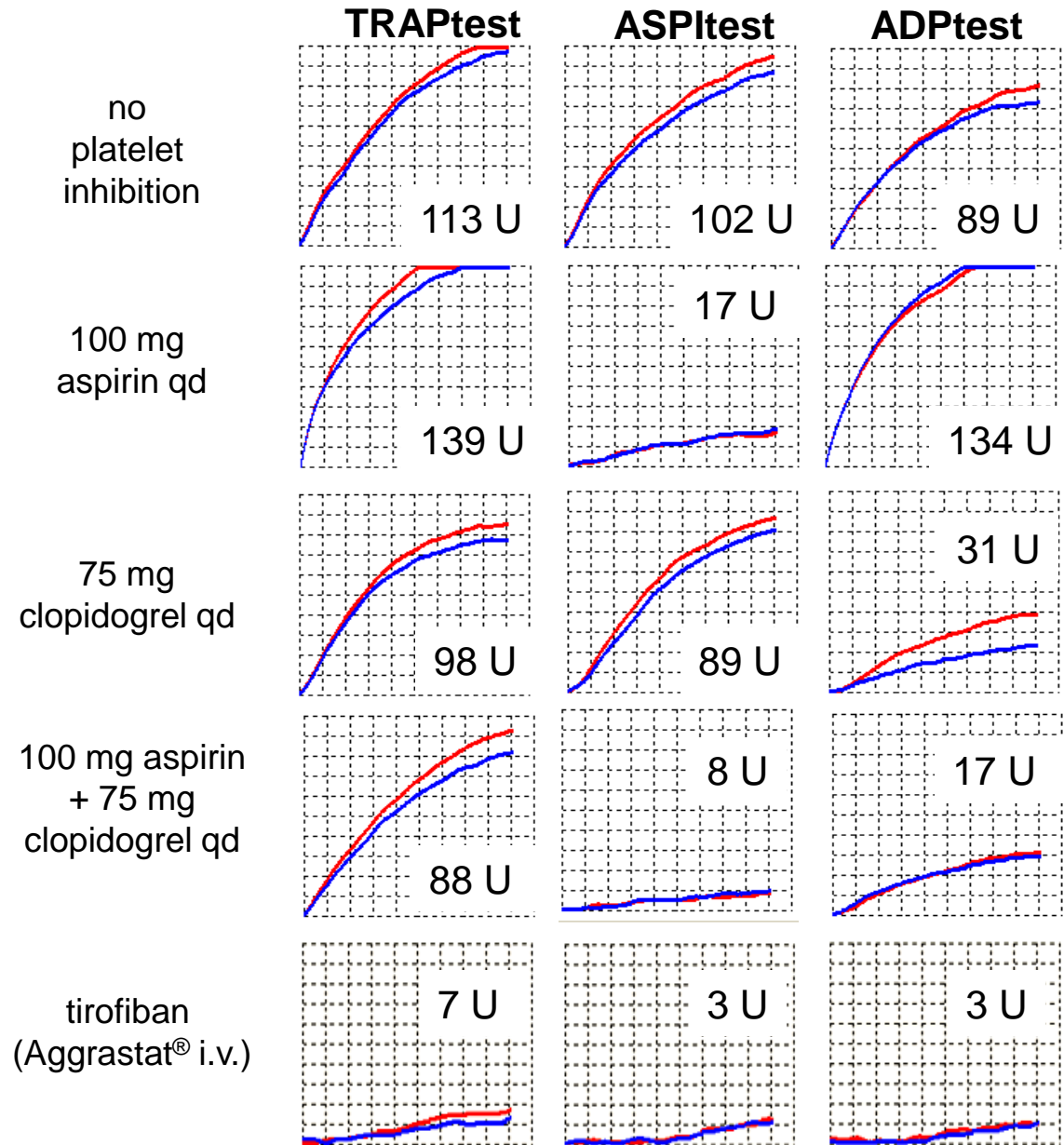
Clinical example 1

A patient with three-vessel disease received a stent in the LAD.

A stent thrombosis occured 6 days after the implantation during therapy with 75 mg clopidogrel and 100 mg of Aspirin.
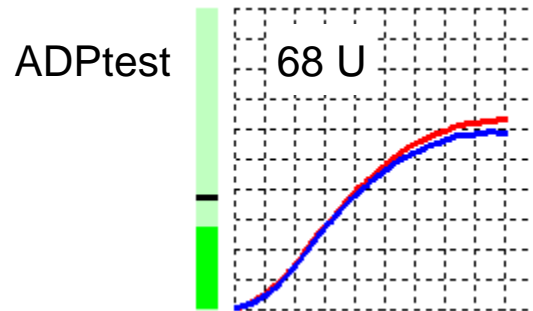
ADPtest   68 U   →low response to clopidogrel (ADPtest > 45 U)

→ patient was switched to 150 mg clopidogrel
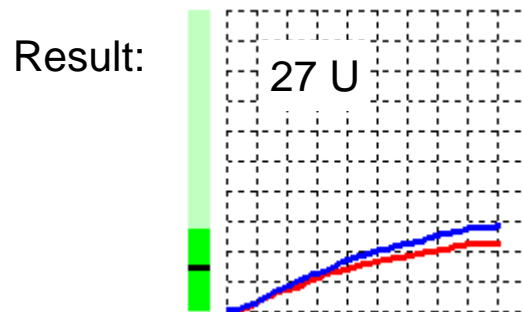
Result: 27 U   →response to 150 mg clopidogrel (ADPtest < 45 U)

Case from Dr. I. Paskaleva, University National Heart Hospital - Sofia

Clinical example 2

A PCI patient with a low response to clopidogrel 75 mg. The patient was switched to Clopidogrel 150 mg – without any effect. In contrast the patient showed an excellent response to 10 mg of Prasugrel.

Clopidogrel 75 mg   Clopidogrel 150 mg   Prasugrel 10 mg
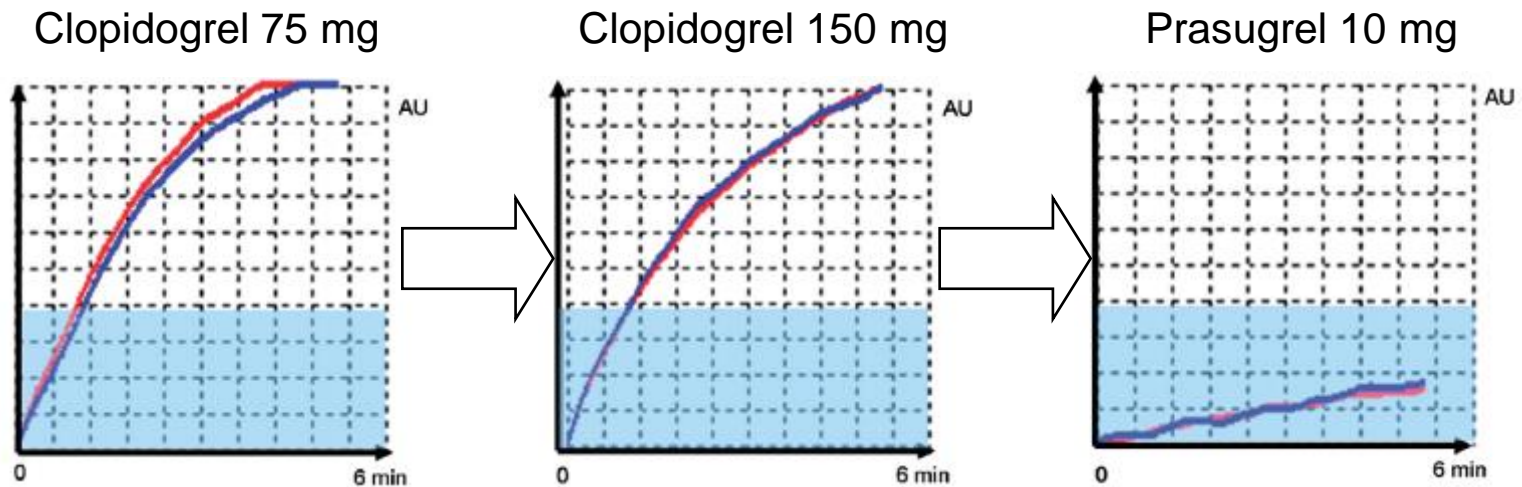
Schuhmann et al. Platelets. 2009 Nov;20(7):498-504. Cardiology, Munich University Clinic.

Clinical example 3

A patient underwent vascular surgery. Post-operatively the patient had a thrombocytosis (1 million platelets / µl) and developed an arterial thrombosis.

100 U
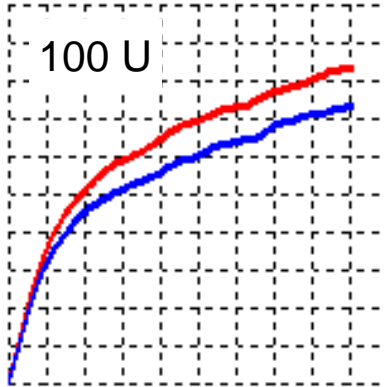
→ aspirin non-response due to enhanced platelet turnover
→ consider increasing aspirin dose to 2 x 100 mg / day
   (or 3 x 100 mg / day if necessary)

69 year old patient, three months after NSTEMI with PCI becomes unconcious.

CT after admission:
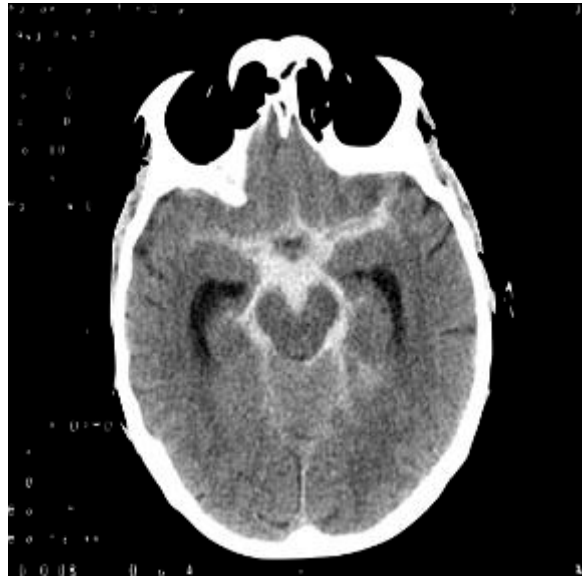
→ extensive subarachnoid bleeding

→ anti-platelet agents were stopped

Treatment with platelet concentrate transfusion, desmopressin and cyclocapron and placement of a drainage.

Case by Niklas Jonsson, Neurological ICU, Karolinska University Clinic, Solna

After 6 days: still bleeding problems, situation very critical.

Laboratory: Hb 9.3, platelet count 263.000, aPTT 41 sec, INR 1.0

Platelet function?

ASPItest	ADPtest	TRAPtest

96 U → platelet activation via COX is intact

92 U → platelet activation via ADP is intact

14 U → platelet activation via PAR-1 is absent
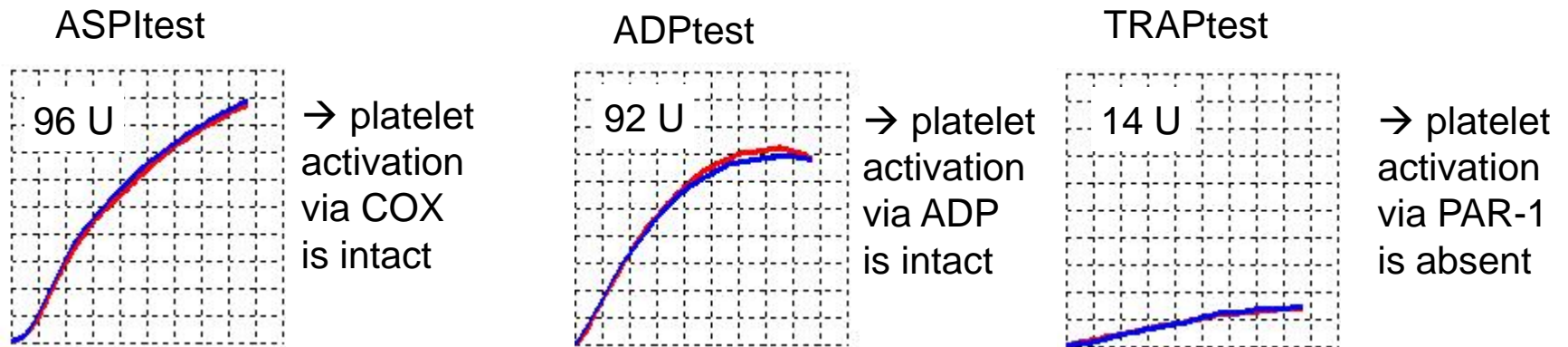
→patient was in a phase III trial with Vorapaxar (TRACER)
→ thrombin receptor antagonist with a half-life of 100-200 h (!)

→the TRACER study was stopped in January 2011 due to an excess in cerebral bleeding

→the TRA-2P study with the same anti-platelet agent is still ongoing

Case by Niklas Jonsson, Neurological ICU, Karolinska University Clinic, Solna

Switch of anti-platelet therapy after neuroradiological intervention

59 year old patient with a SAB due to a wide-neck aneurysm of the ACI.

Intervention using coils and a stent.

After the intervention i.v. therapy using aggrastat.

After several days loading with 300 mg clopidogrel and 500 mg aspirin at 0:00, aggrastat stopped at 4:00.

→ stent thrombosis and large cerebral infarction

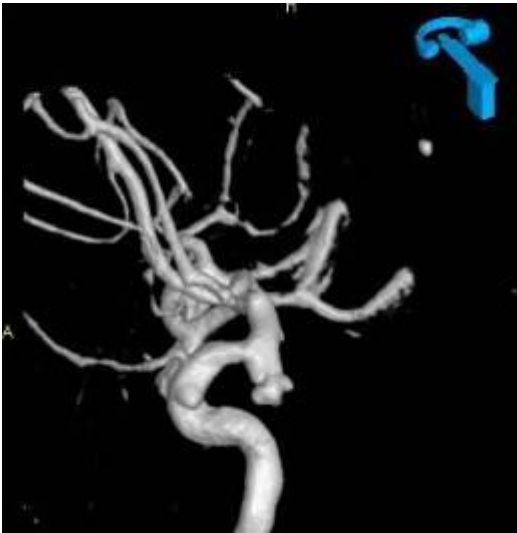# Switch of anti-platelet therapy after neuroradiological intervention

59 year old patient with a SAB due to a wide-neck aneurysm of the ACI.

Intervention using coils and a stent.

After the intervention i.v. therapy using aggrastat.

After several days loading with 300 mg clopidogrel and 500 mg aspirin at 0:00, aggrastat stopped at 4:00.

→ stent thrombosis and large cerebral infarction

Cause?

ASPItest

146 U

ADPtest

124 U
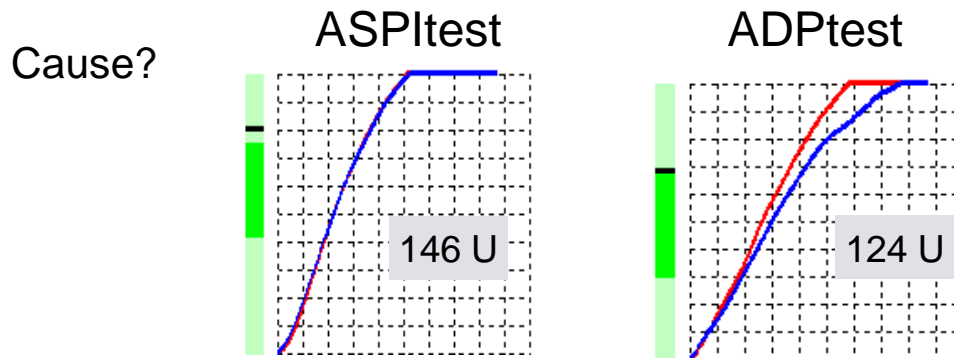
→ patient was intubated and was loaded via the gastric tube:
Aspirin and clopidogrel was not absorbed (pharmacokinetic non-response to anti-platelet treatment)

Platelet Reactivity After Clopidogrel Treatment Assessed With Point-of-Care Analysis and Early Drug-Eluting Stent Thrombosis

Dirk Sibbing, MD, Siegmund Braun, MD, Tanja Morath, MS, Julinda Mehilli, MD, Wolfgang Vogt, MD, Albert Schömig, MD, Adnan Kastrati, MD, Nicolas von Beckerath, MD

Cumulative incidence of stent thrombosis (%)

$P < 0.0001$

n=1608

OR 9.4

Low-responders

Normal-responders

Days after enrollment
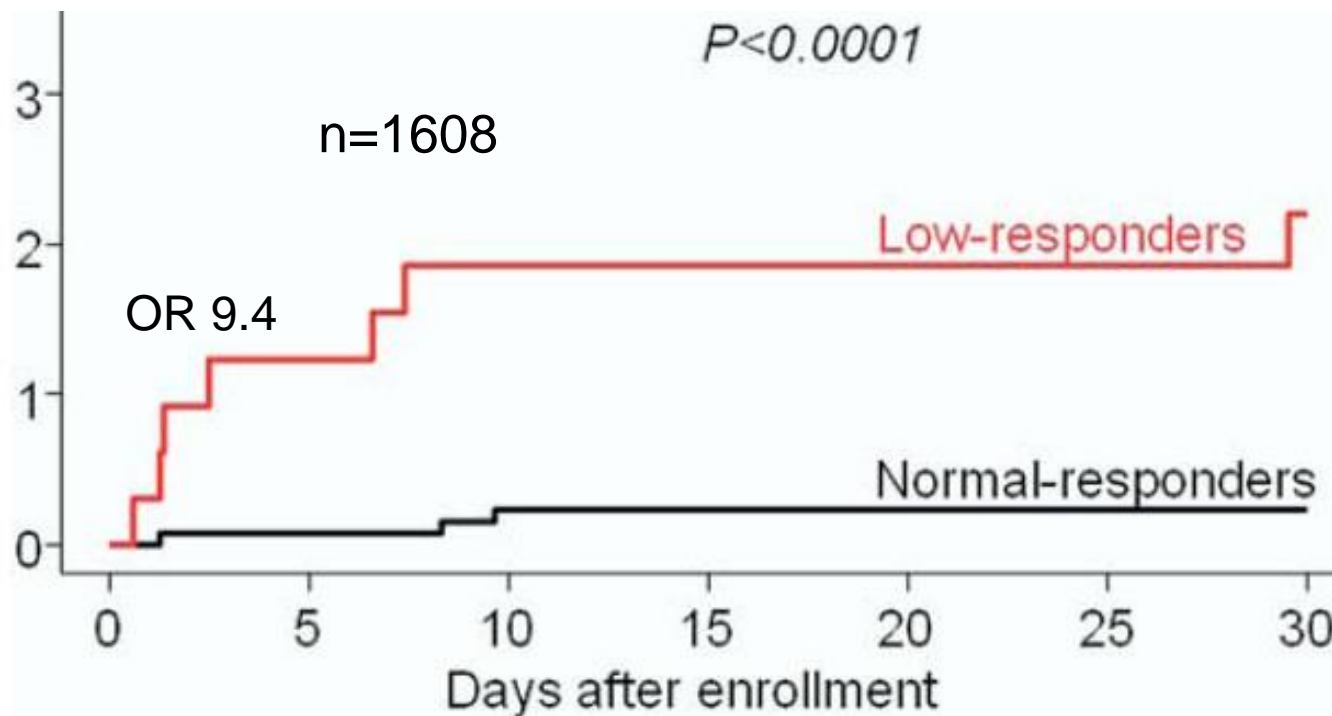
5-10 x increased risk for ST, q-wave MI and stroke for clopidogrel low-responders (20% of the patients)

very low risk for clopidogrel „responders" (80% of the patients)

ORIGINAL ARTICLE

Multiple electrode aggregometry predicts stent thrombosis better than the vasodilator-stimulated phosphoprotein phosphorylation assay

J. M. SILLER-MATULA,* G. CHRIST,† I. M. LANG,‡ G. DELLE-KARTH,‡ K. HUBER§ and B. JILMA*

*Department of Clinical Pharmacology, Medical University of Vienna, Vienna; †5th Medical Department, Kaiser-Franz-Josef Hospital, Vienna; ‡Department of Cardiology, Medical University of Vienna, Vienna and §3rd Medical Department of Cardiology and Emergency Medicine, Wilhelminenhospital, Vienna, Austria
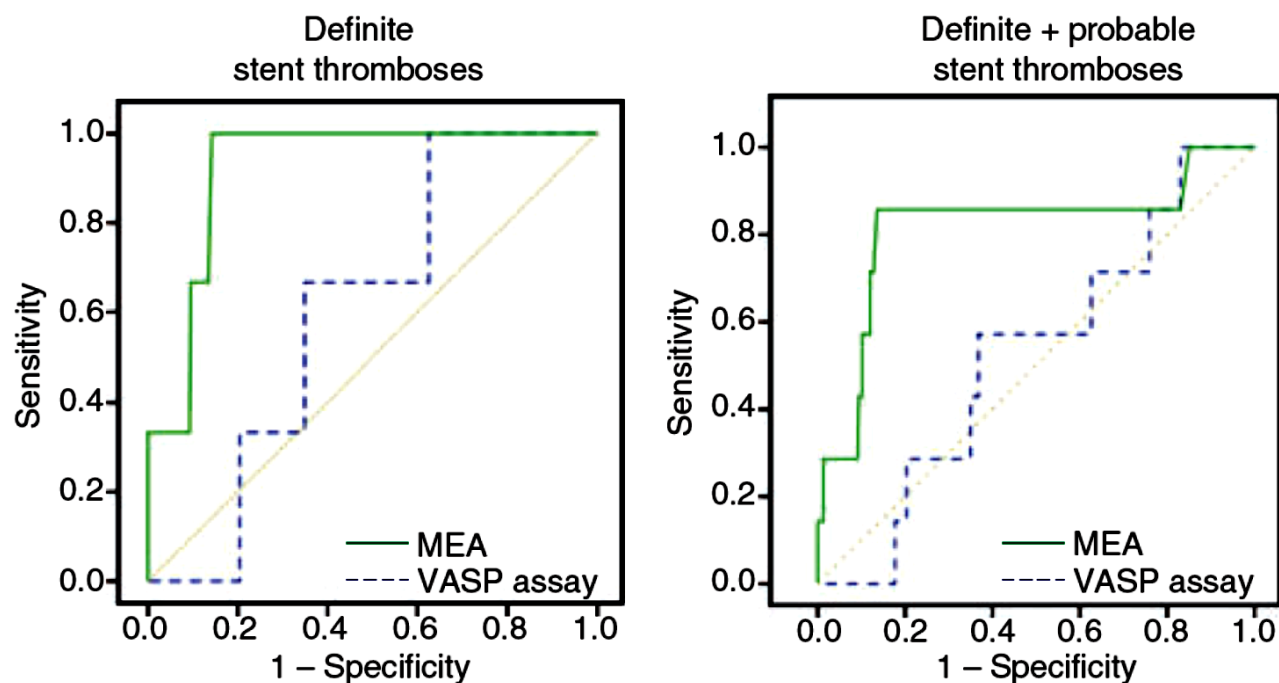
Consensus Paper on ADP receptor antagonist monitoring

Consensus and Future Directions on the Definition of High On-Treatment Platelet Reactivity to Adenosine Diphosphate

Laurent Bonello, MD,* Udaya S. Tantry, PHD,§§ Rossella Marcucci, MD, PHD,‖
Ruediger Blindt, MD,# Dominick J. Angiolillo, MD, PHD,‖‖ Richard Becker, MD,¶¶
Deepak L. Bhatt, MD, MPH,## Marco Cattaneo, MD,¶ Jean Philippe Collet, MD, PHD,‡
Thomas Cuisset, MD,† Christian Gachet, MD, PHD,§ Gilles Montalescot, MD, PHD,‡
Lisa K. Jennings, PHD,*** Dean Kereiakes, MD,††† Dirk Sibbing, MD,**
Dietmar Trenk, PHD,†† Jochem W. Van Werkum, MD, PHD,‡‡ Franck Paganelli, MD,*
Matthew J. Price, MD,‡‡‡ Ron Waksman, MD,§§§ Paul A. Gurbel, MD,§§
for the Working Group on High On-Treatment Platelet Reactivity

Table 2

Studies Linking High On-Treatment Platelet Reactivity to Ischemic Events Based on ROC Curve With a Specific Cutoff Value

Study (Ref. #)	Assay	End Point	AUC	Odds Ratio
Gurbel et al. (69)	LTA	2-year post-PCI MACE	0.77 0.78	3.9 3.8
Blindt et al. (62)	VASP-PRI	6-month ST	0.79	1.16
Marcucci et al. (75)	VerifyNow P2Y12 assay	1-yr CV death and nonfatal MI	0.66	2.38 CV death 2.76 nonfatal MI
Sibbing et al. (80)	Multiplate analyzer-ADP	30-day ST	0.78	12.0
Cuisset et al. (81)	LTA	1-month ST	0.69	5.8
Breet et al. (82)	LTA VerifyNow P2Y12 assay Plateletworks	1-yr death, MI, ST, and stroke	0.63 0.62 0.62 0.61	2.09 2.05 2.53 2.22

→ best predictivity for Multiplate

Multiplate analysis and prediction of bleeding
Cardiac surgery in patients pre-treated with thienopyridines

- evaluation of 87 patients undergoing cardiac surgery under clopidogrel therapy

- wide variation of ADP induced aggregation

- when ADPtest was ≤ 31 U risk of excessive bleeding was 29%

- when ADPtest was > 31 U risk of excessive bleeding was 8% ($p < 0.05$)

Ranucci M et al. Multiple electrode whole-blood aggregometry and bleeding in cardiac surgery patients receiving thienopyridines. Ann Thorac Surg. 2011 Jan;91(1):123-9.
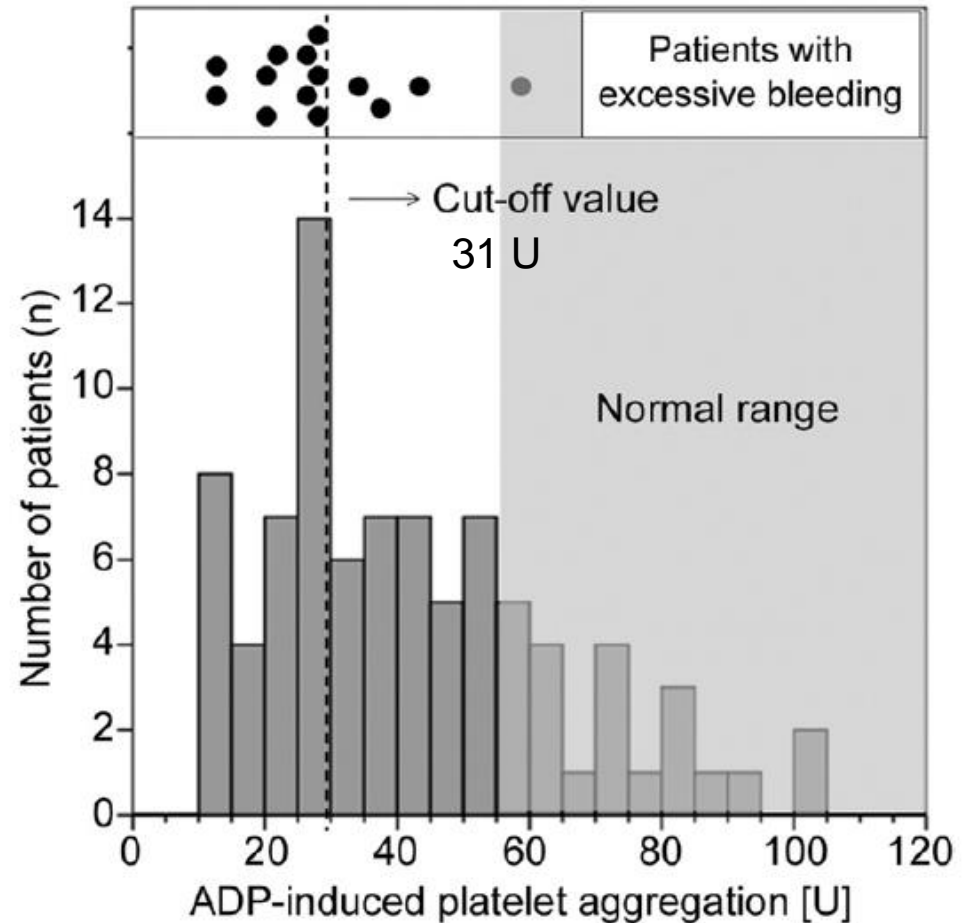
Multiplate analysis and prediction of bleeding
Risk of TIMI major bleeding in patients after PCI

Sibbing D et al. J Thromb Haemost. 2010;8(2):250-6.
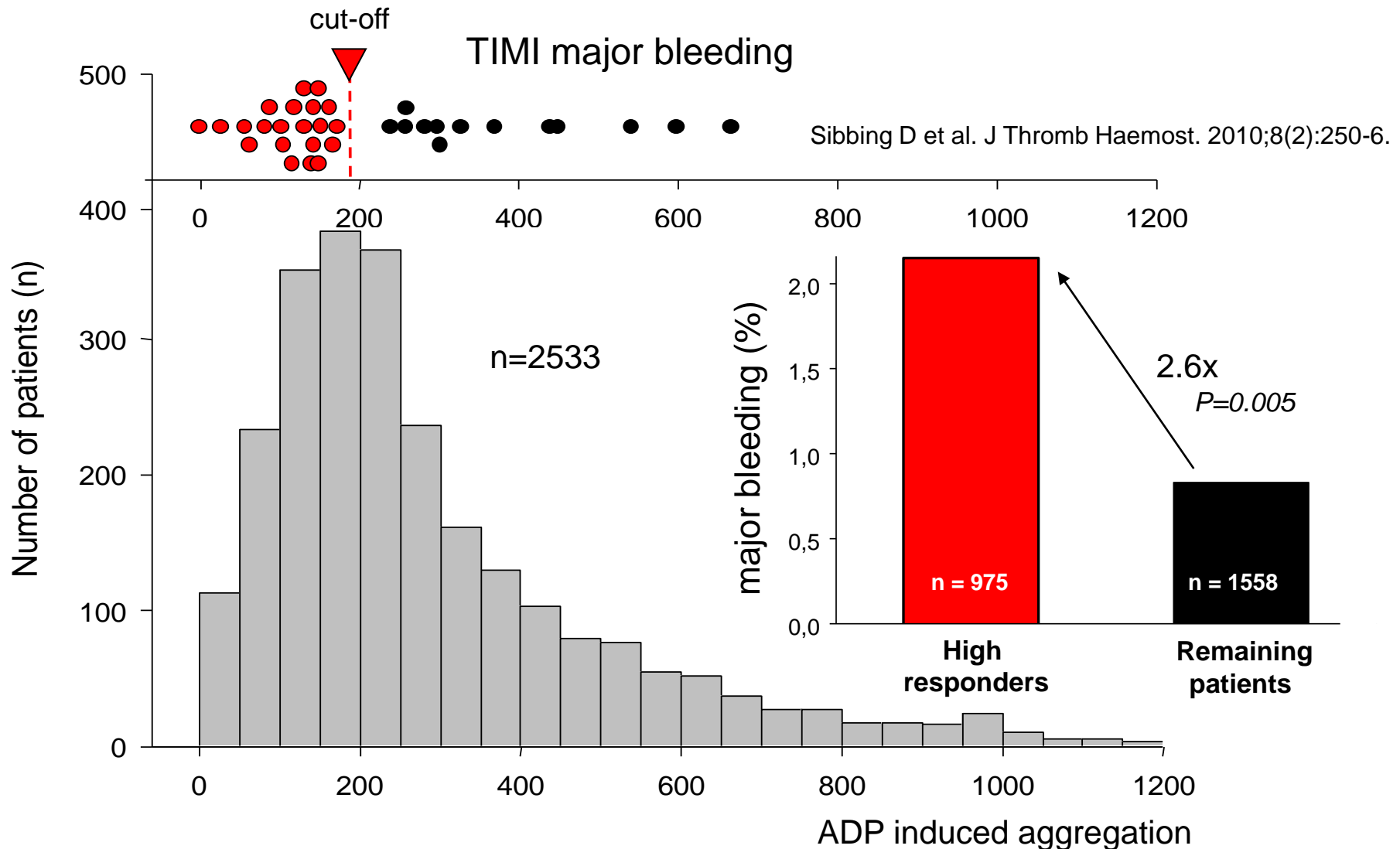
→ 2.6 fold increased risk for TIMI major bleeding after PCI when ADPtest < 19 U

Accumetrics Verifynow
No / modest prediction of early events in the 3 largest prospective studies

Patients with and without high on-treatment platelet reactivity receiving standard-dose clopidogrel

Cumulative Incidence of Primary End Point, %

On-treatment platelet reactivity
—— High
----- Not high

Price et al.
JAMA 2011
n= 1691

Hazard ratio, 1.68; 95% CI, 0.76-3.72; $P = .20$

Days

No RPR (PRU <240)
RPR (PRU ≥240)

CV death-free Survival

Marcucci et al.
Circulation 2009
n= 683

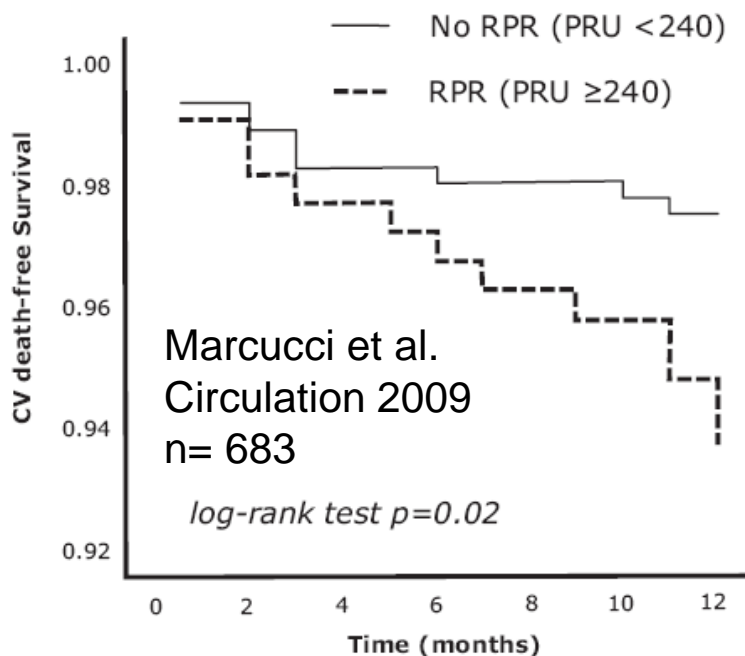*log-rank test p=0.02*

Time (months)

Figure 2. Survival free from cardiovascular (CV) death in patients with and without PRU ≥240.

VerifyNow P2Y12

Event Rate, %
Combined Primary End Point

Breet et al.
JAMA 2010
n=1052

Log-rank $P <.001$

Time, d

in all 3 studies the Kaplan-meier curves divide only late

Siemens PFA-100
No predictivity during the first 190 days after PCI

On-treatment platelet reactivity
— High
- - - Normal

INNOVANCE

n=588

Log-rank $P = .02$
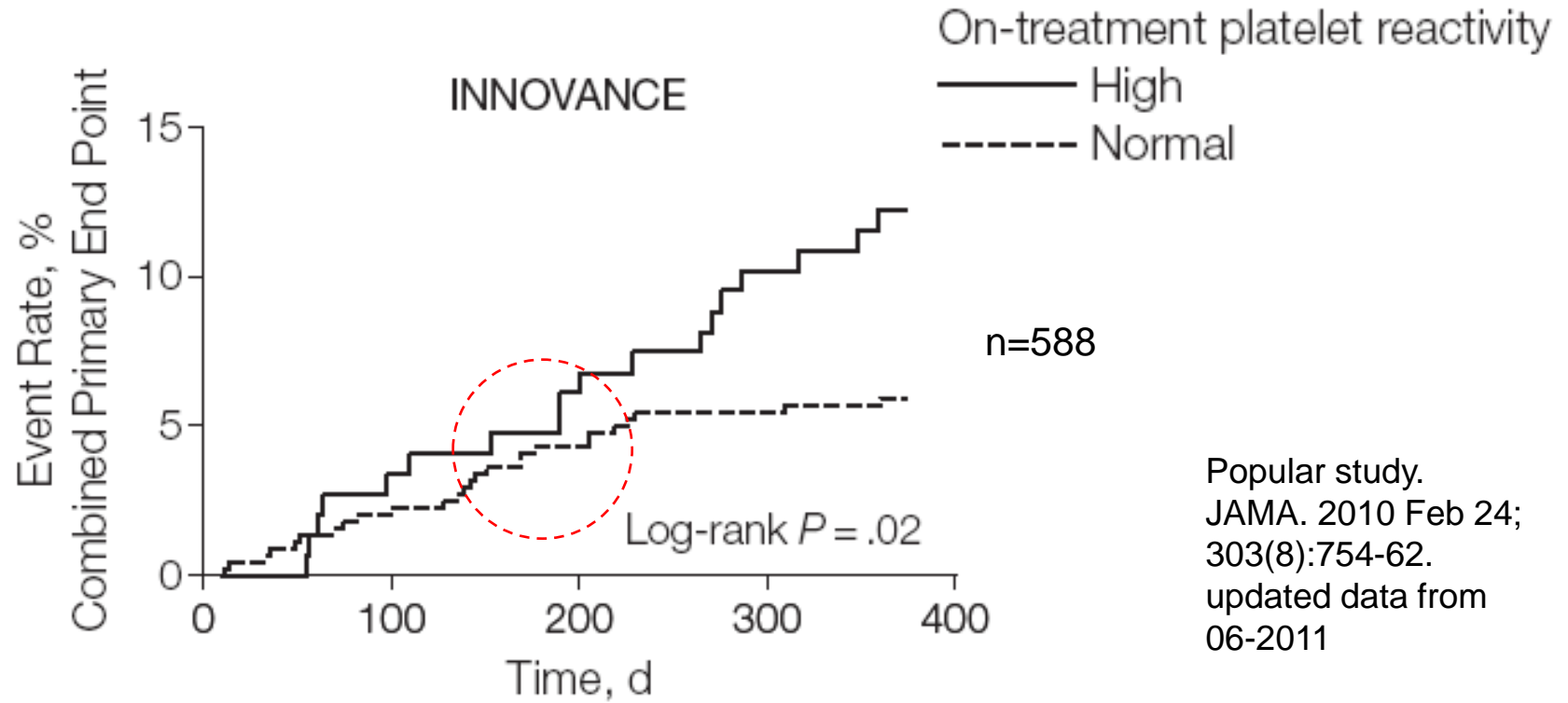
Popular study.
JAMA. 2010 Feb 24;
303(8):754-62.
updated data from
06-2011

In the only clinical study so far on the new PFA-100 P2Y cartridge clopidogrel <u>low responders</u> according to PFA-100 had less stent thromboses, less trokes and less target vessel revascularisations compared to clopidogrel responders. The Kaplan-Meier curves divided only after 190 days, which makes any use of this cartridge for the clinical management absurd.

In contrast to Verifynow and PFA-100 Multiplate is capable to predict early adverse events

Sibbing et al, TH 2009

n=1608

Low responders

Normal responders

Definite or probable ST (%)

days after enrollment
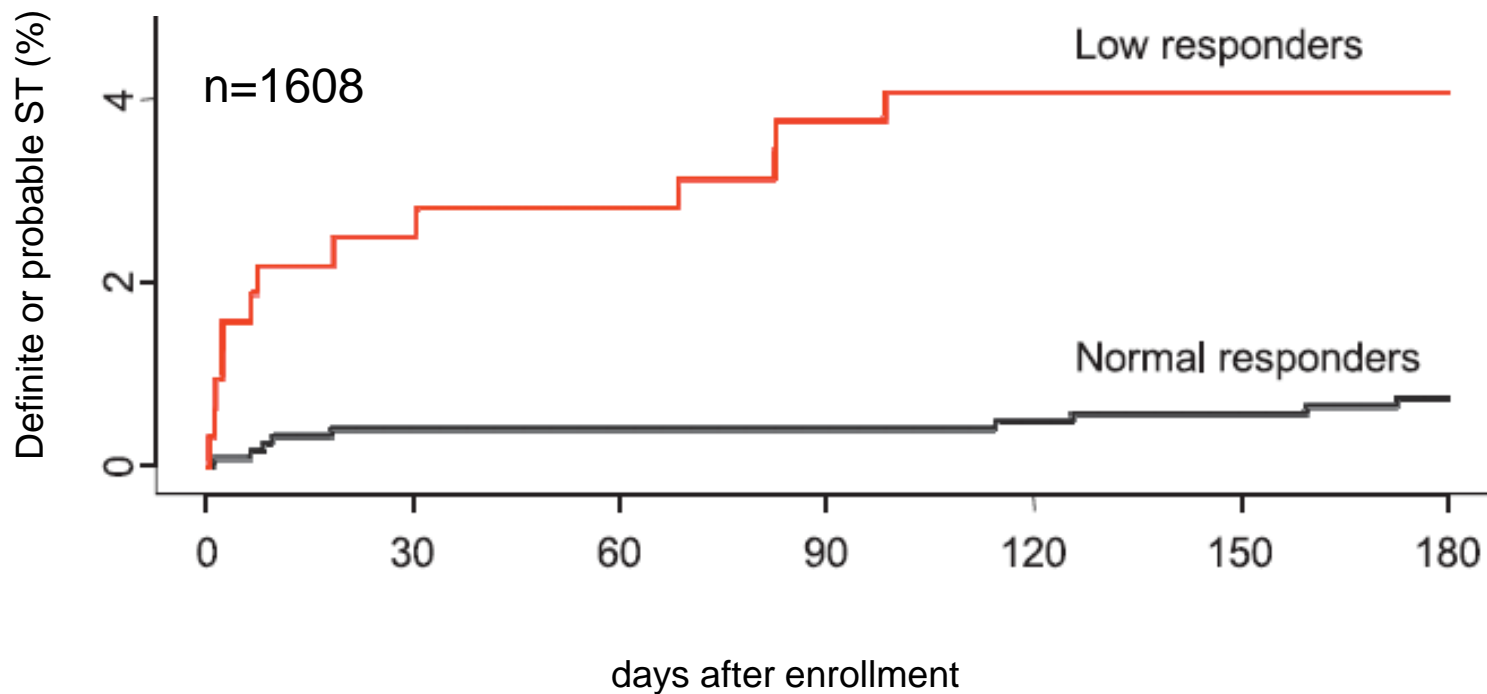
Conclusions

Clopidogrel and Aspirin are potent anti-platelet medications, however they do not always act properly

Prasugrel and Ticagrelor are two new ADP receptor antagonists that have advantages and disadvantages vs. Clopidogrel.

Platelet function testing can provide clinically relevant information for individualising anti-platelet therapy.

Multiplate analysis is the most widely used method for tailoring ADP receptor antagonist therapy in Europe.

The predictive value of Multiplate for bleeding events, stent thrombosis and stroke has been shown in large clinical studies.
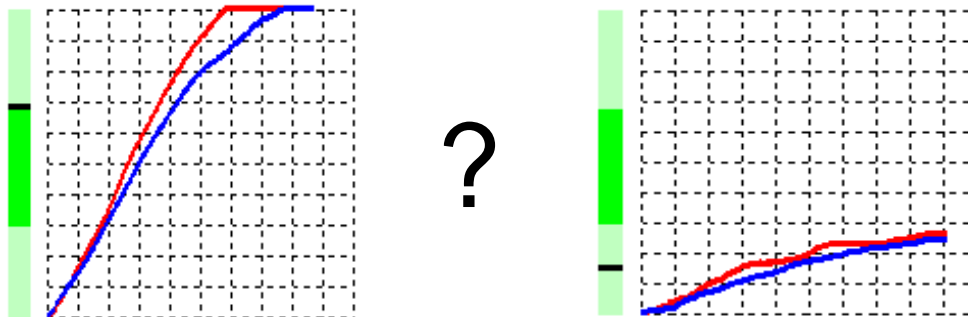
Thank you very much for your attention!

ORIGINAL RESEARCH

Monitoring of Clopidogrel-Related Platelet Inhibition: Correlation of Nonresponse with Clinical Outcome in Supra-aortic Stenting

S. Müller-Schunk
J. Linn
N. Peters
M. Spannagl
M. Deisenberg
H. Brückmann
T.E. Mayer
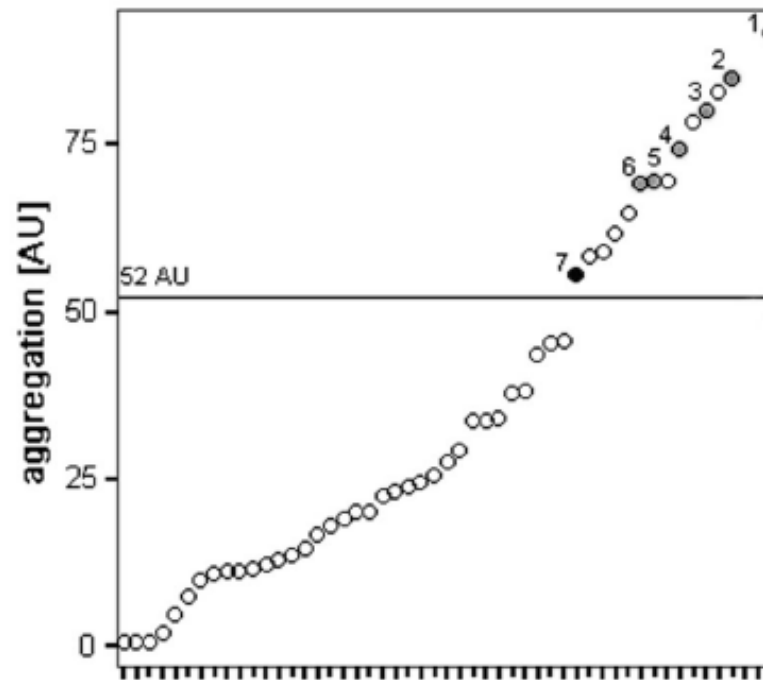
Accumetrics Verifynow
Principle

Mixing chamber

Light source

Increase in light transmittance with agglutination of beads; rate and extent of change measured

GP IIb/IIIa integrin

Fibrinogen

Platelets in whole blood activated by specific agonist in mixing chamber

+

Fibrinogen-coated beads

→

Agglutinated beads fall out of the solution

Mixing Chambers/ Detection Well
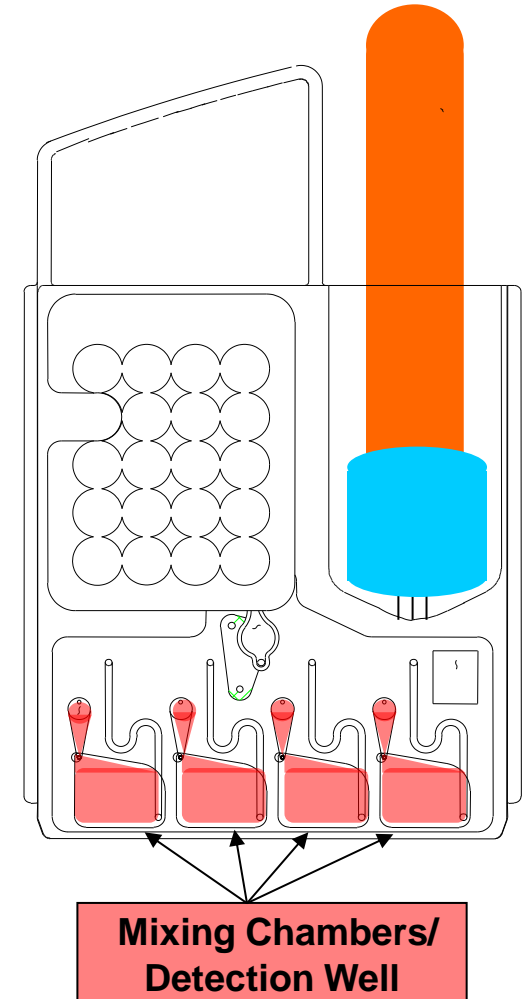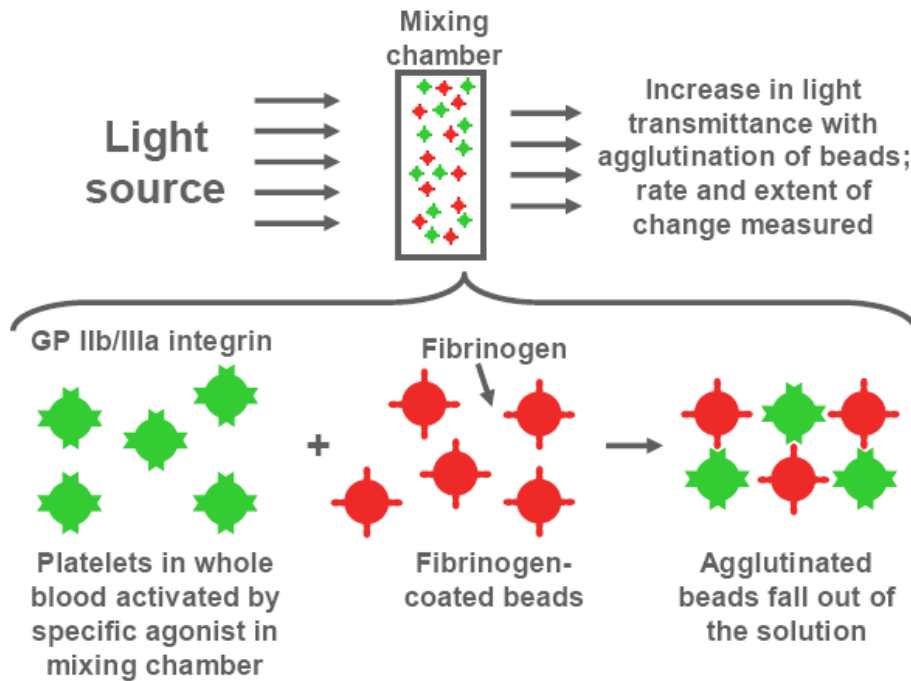
GRAVITAS Patient Flow

5429 patients screened with VerifyNow P2Y12
12-24 hours post-PCI

2214 (41%) with high residual platelet reactivity (PRU ≥ 230)

3215 (59%) without high residual platelet reactivity (PRU < 230)

Random selection

Clopidogrel High Dose N=1109

Clopidogrel Standard Dose N=1105

Clopidogrel Standard Dose N=586

Non-Randomized Comparison

GRAVITAS observational substudy:
The patients determined as clopidogrel "resistant" using the Verifynow had no significantly increased risk for adverse events!

Price M et al. AHA 2010: Late breaking trial
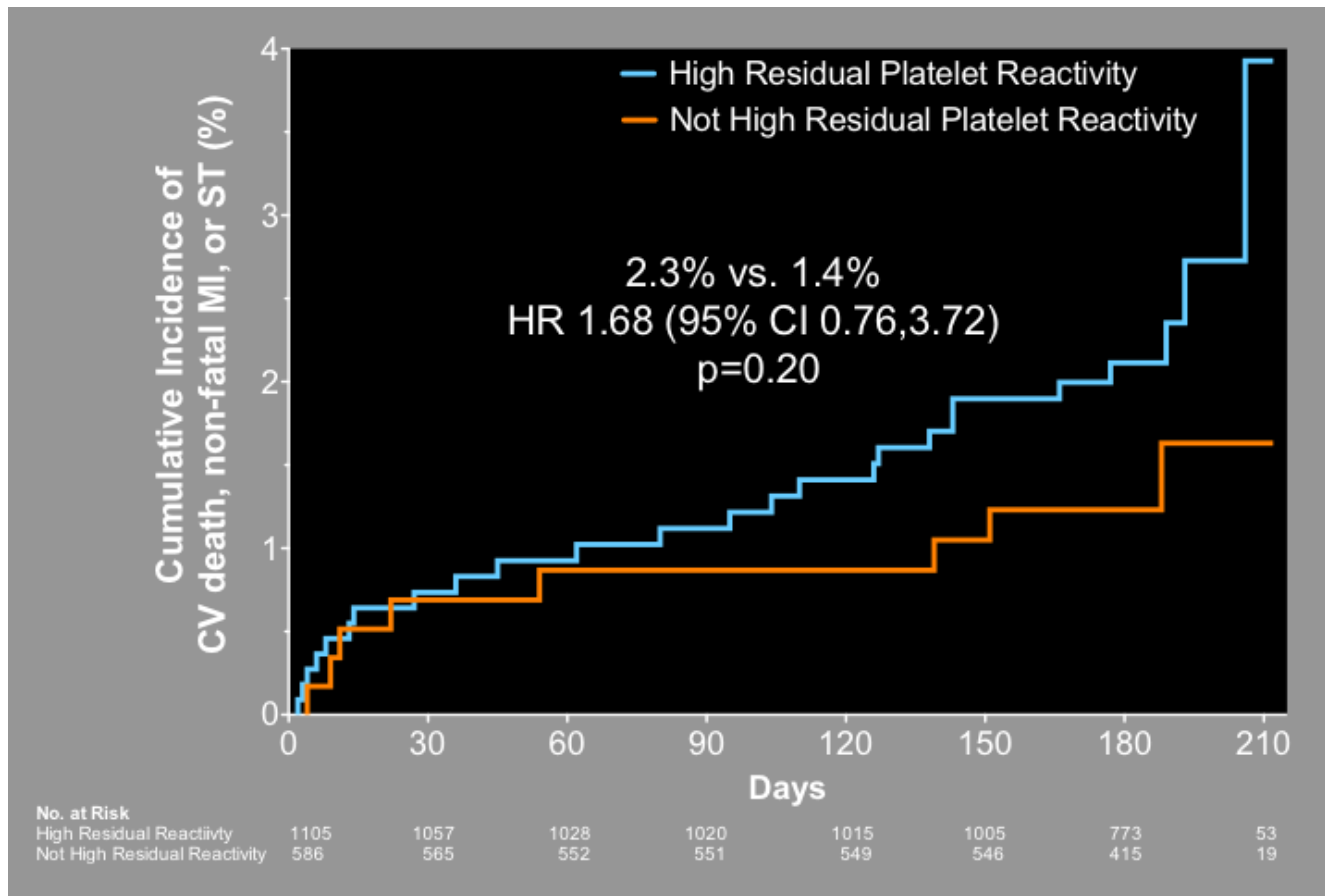
- **non-significant trend towards higher risk over 6 month (HR 1.68; p=0.20)**
- **no difference at all between responders and "resistant" patients according to Verifynow at 30 days.**

Light transmission aggregometry
Principle

- assesses platelet activation by the reduction of optical density of platelet rich plasma

- requires the preparation of platelet rich and platelet poor plasma

- laborious and time-consuming

Platelet Rich Plasma (PRP)

+ Aggregating Reagent → Aggregate Clumping

Baseline Light Transmission

Increased Light Transmission
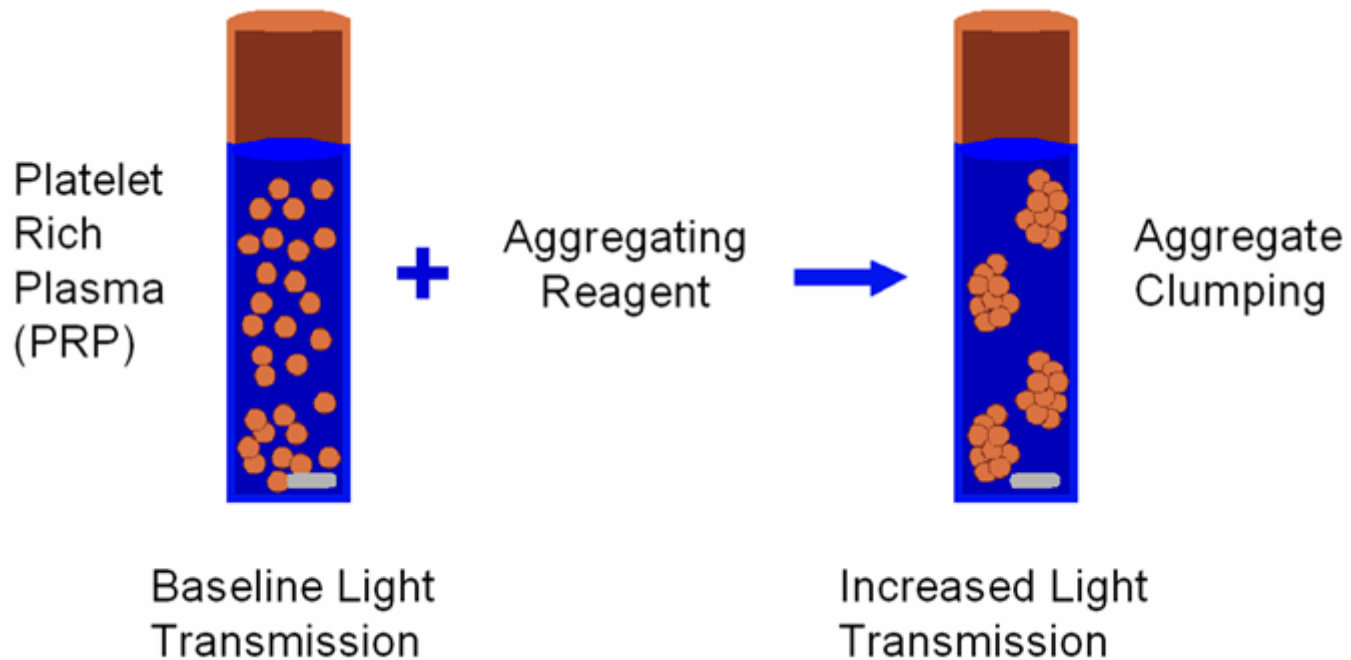
Light transmission aggregometry
Standardisation problems: Cut-Offs for Clopidogrel „Resistance"

| Study	Instrument	ADP	Centrifugation	Cut-Off
Matetzky S et al. Am J Cardiol. 2008 Sep 1;102(5):524-9.	PACKS-4 (Helena)	5 µM	no information	**80% maximal aggregation**
Gurbel PA et al. Platelets. 2008 Dec;19(8):595-604.	490-4D (Chronolog)	5 µM	120 g for 5 minutes	**46% maximal aggregation**
Hochholzer W et al. J Am Coll Cardiol. 2006 Nov 7;48(9):1742-50.	PAP-4 (Bio/Data)	5 µM	750 g for 2 minutes	**14% late aggregation**
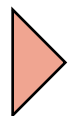
▷ **5 µM ADP-induced aggregometry:**
cut-offs between 14% and 80% are applied for
detection of clopidogrel „resistance"

In contrast to Verifynow and PFA-100 Multiplate is capable to predict bleeding in clopidogrel-treated patients

Verifynow:

Popular study (JAMA 2010) n=1052: no prediction of bleeding

Eng, M. et al (ACC 2011) n=859: no prediction of bleeding

Gravitas study (JAMA 2011) n=1691: no prediction of bleeding

Ricottini E. et al (ACC 2011) n=310: prediction of bleeding

→ only 1 out of 4 studies positive (the smallest)

PFA-100:

Popular study (JAMA 2010) n=588: no prediction of bleeding

Multiplate:

Sibbing D. el al. (JTH 2010) n=2533: prediction of bleeding

Ranucci M. et al. (ATS 2011) n=87 (CABG): prediction of bleeding

Which ADP receptor antagonist to choose ?

Pharmacology   **costs / year**

Plavix.
(clopidogrel bisulfate) 75mg tablets

pro-drug, good safety and side-effect profile
1x daily application
20 years experience
but: ~ 20% low-responders

generic clopidogrel:
~ 50 €

Efient
prasugrel tablets

pro-drug, high efficacy (very few low-responders)
1x daily application
but: 4 x higher bleeding risk compared to clopidogrel

~ 1000 €

BRILIQUE. 90mg
ticagrelor

direct antagonist, high efficacy (very few low-responders)
2x daily application
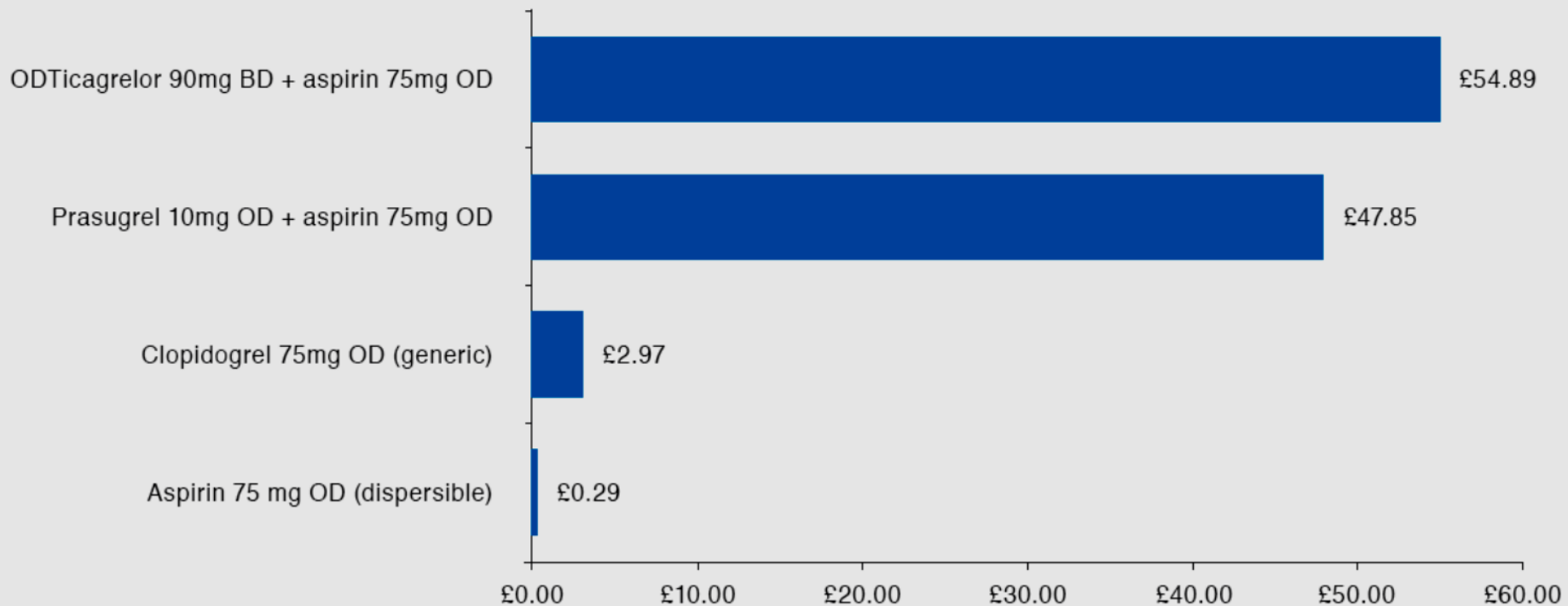but: 14% dyspnea, more bleeding compared to clopidogrel

~ 1100 €

Which ADP receptor antagonist to choose ?
Costs of Anti-Platelet Therapy

How much does it cost?

Cost of 28 days treatment (eMIMS, Drug Tariff January 2011)

- ODTicagrelor 90mg BD + aspirin 75mg OD — £54.89
- Prasugrel 10mg OD + aspirin 75mg OD — £47.85
- Clopidogrel 75mg OD (generic) — £2.97
- Aspirin 75 mg OD (dispersible) — £0.29

Source: New drug evaluation: Ticagrelor, 01-2011, Regional Drug and Therapeutics Centre, NHS

Will Prasugrel and Ticagrelor eliminate the need for platelet function testing?
4 x higher bleeding risk with Prasugrel vs. Clopidogrel

TRITON-TIMI 38.
NEJM 2007;357:2001-15.

p<0.001

4x

Y-axis: CABG-related TIMI major bleeding (0, 5, 10, 15, 20, 25)

clopidogrel
n=189

prasugrel
n=179
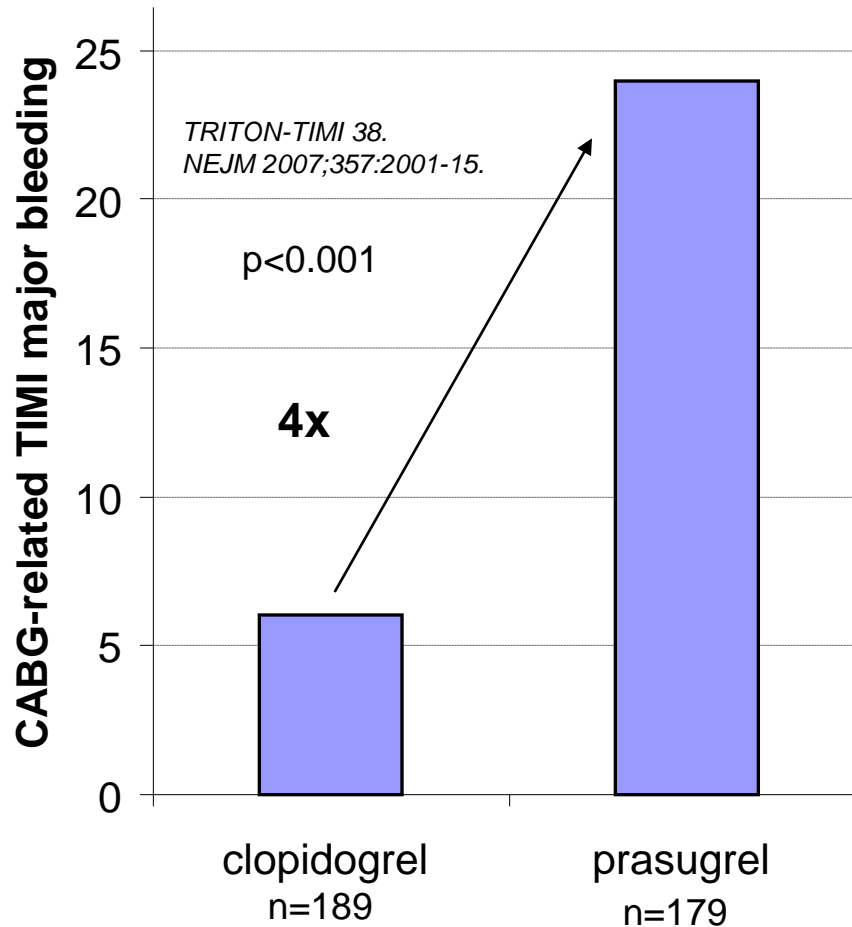
EuroPCR Session
"Testing antiplatelet reactivity in clinical practice"
4 of 5 presentations included data generated with Multiplate

Testing antiplatelet reactivity in daily practice
Thursday 19 May, 2011 - Room 253 - 10:30 - 12:00

Routine tailoring of antiplatelet therapy after coronary stent implantation has the potential to eradicate early definite stent thrombosis in compliant patients
Speakers: Günter Christ

Peri-procedural variations of platelet function in patients undergoing PCI
Speakers: Fabio Mangiacapra

Individualising clopidogrel therapy according to the multiplate aggregometry test reduces the ischemic complications in patients after PCI and stenting
Speakers: Dobri Hazarbasanov

Platelet aggregation in stable angina patients treated with aspirin and clopidogrel is modulated by a 6.3 / 6.7 kb polymorphism of the platelet Alpha2A-adrenergic receptor
Speakers: Aaron. J Peace

Real-time clopidogrel gain-of-function and loss-of function genotype screening with NanosphereVerigene2C19/CBSNucleic Acid
Speakers: Matteo Tebaldi