

Arizona Society of Pathologists 2013
Spring Conference

*Genotype Guided Clopidogrel (Plavix) Dosing in
Patients with Stable Cardiovascular Disease*

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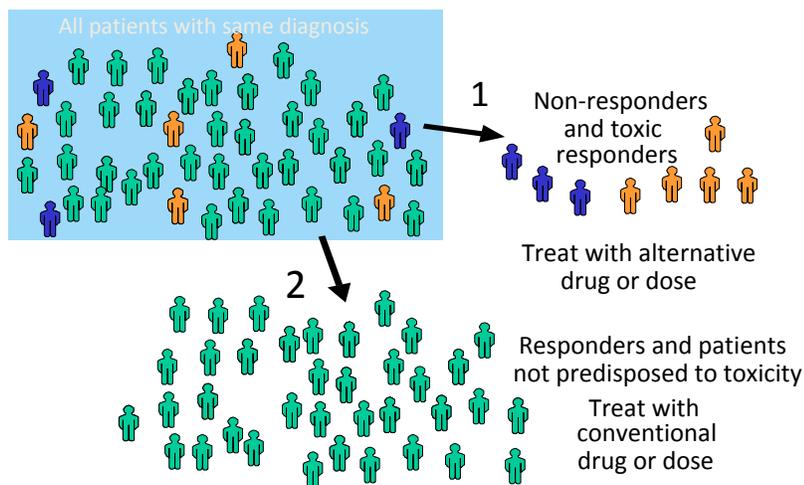
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ELEVATE-TIMI 56 (Escalating Clopidogrel
by Involving a Genetic Strategy -
Thrombolysis in MI 56)

In medicine same drug/dose may not work for all patients

- Same cancer (e.g. breast cancer) medication may have different responses in different patients
- Hypertension drugs works in 10-30 % of patients
- Asthma drugs works in 40-70 % of patients
- Annually >2 million adverse drug reactions in US. ~100,000 death; 4th leading cause. (Ingelman-Sundberg NEJM 2008;358: 637)
- Standard dose based on weight, age, medical history; it may not work or cause side effects

One size does not fit all



Why one size does not fit all?

Inter-individual Variability in Drug Disposition
and Action

Therapeutic drug monitoring (TDM)

- TDM measures blood concentration of a drug
- Indicative of an individual's ability to absorb, distribute, metabolize and excrete the drug
- It does not take into consideration of variability of pharmacodynamic responses such as sensitivity to its cellular target receptors in an individual

Factors Contributing to Interindividual Variability in Drug Disposition and Action

- Age
- Weight
- Gender
- Concomitant Diseases
- Concomitant Drugs
- Social factors
- GENETICS



**PERSONALIZED
MEDICINE**

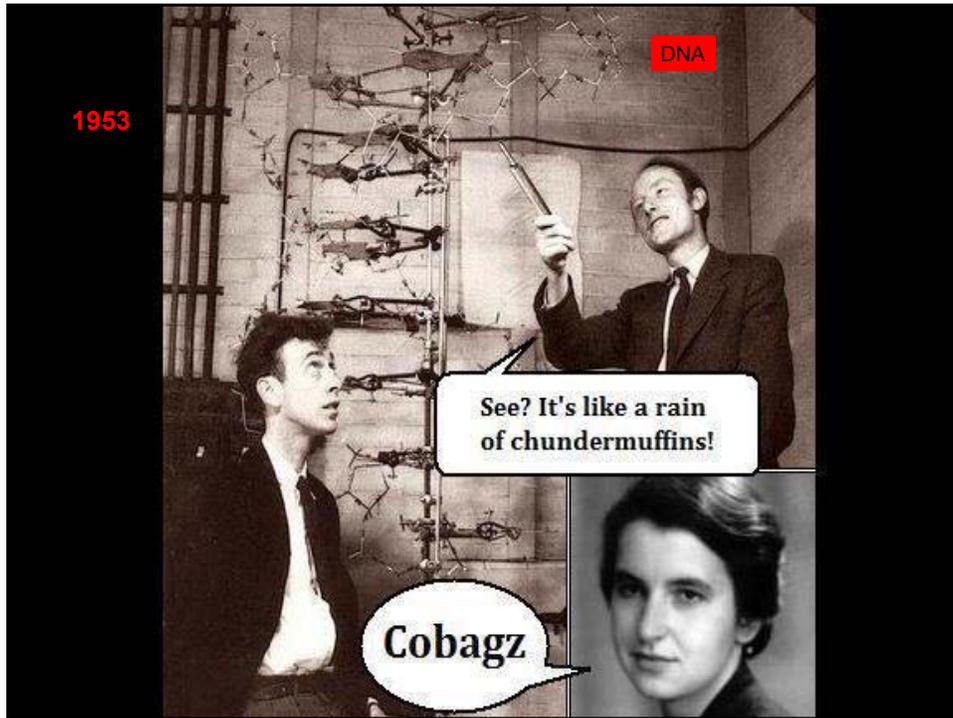
or

**Individualized
Drug Therapy**

Individualized drug therapy

Genomic Medicine

Promise of pharmacogenomics in drug therapy



DNA and Human Genome

- It took only 50 yrs (1953 ~ 2003) to progress from the discovery of DNA double helix structure to completion of sequencing of 3 billion base pairs in a human sperm or an egg cell.
- Imagine what will bring in next 50 yrs?

The Foundation of Pharmacogenomics:

Differences in the genetic code between people

• **Mutation:** Difference in the DNA code that occurs in less than 1% of population and often associated with rare diseases

E. g: Cystic fibrosis, sickle cell anemia, Huntington's disease

• **Allele:** A slight difference in gene sequence results in a variation in <1 % of population, but not associated with a disease.

–Variation due to an allele of a gene is called heterozygous variant

–Variation in two alleles (same location) of a gene is known as homozygous variant

• **Polymorphism:** When an allele(s) is present in more than 1% of the population

–A single polymorphism is less likely to be the main cause of a disease

–Often have no visible clinical impact

E.g: Hair or eye color

Single nucleotide *polymorphism* (SNP)

- It refers to a difference of single nucleotide between sequences of individuals in the same location of a gene and is present in > 1% in a population
- Estimated 10,000,000 SNPs differentiate humans from each other within the 3 billion base pair genome

Consequences of Polymorphisms

- An SNP in a *noncoding* sequence may result in altered abundance of a transcript (mRNA) or a transcript with altered stability.
- A polymorphism in a *coding* sequence may produce a protein with altered activity due to substitution by a different amino acid
- No consequence

Cytochrome P 450

The super-family of cytochrome P450 enzymes has a crucial role in the metabolism of drugs. Exist in most species. In human there are 18 family and subfamilies arising from sequence homologies between them.

E.g:
Family: CYP2

Subfamily: CYP2C

Member: CYP2C19



Wild type allelic designation:
CYP2C19*1*1
Heterozygote carrier (variant):
CYP2C19*1*2
Homozygote carrier:
CYP2C19*2*2

Almost every drug is processed by some of these enzymes. Nomenclature specifies a single P 450 protein indicating the family and subfamily isoform

SNP and Cytochrome P 450

- Slight variation of a gene sequence resulting from SNP in any of these drug metabolizing enzymes may have profound effect on how an individual responds to certain medications
- The drug may become toxic, may or may not work for the specific individual

Clopidogrel (Plavix)

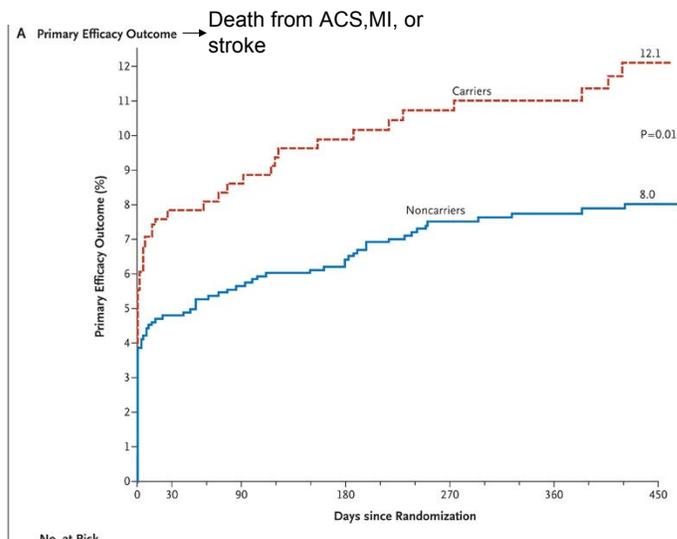
- It ranks 2nd best selling drug in the world and has become standard of care with aspirin for patients with ACS and for patients receiving stents.
- Several studies indicate that significant number of patients may not be getting full benefit of the drug due to common genetic variations in the Cytochrome P450 (CYP) enzyme responsible for its metabolism. In this case it is CYP2C19.

Mechanism of action

- Clopidogrel must be metabolized to active metabolite by CYP enzymes (CYP2C19)
- *Active metabolite* binds to P2Y₁₂ ADP receptor on platelet surface, impeding platelet aggregation and reducing risk of thrombosis

PGx of clopidogrel

Mega et al. NEJM 2009;360:354-62.



JAMA (2010) 304; 1821-1830

- Meta analysis of 9 studies; 9685 patients,
- Coronary artery stents taking plavix
- 1.7 times risk of stent thrombosis with one reduced function allele CYP2C19 gene variants (26.3%)
- 3 times risk of stent thrombosis with 2 reduced function alleles compared to wild type(2.2%)

FDA Black box Warning for Clopidogrel

Some patients may be poor metabolizers of Plavix. They do not effectively convert Plavix to its active form because of low **CYP 2C19** activity. The effectiveness of Plavix as a preventive therapy is reduced.

Tests are available to determine patients' **CYP2C19** status. Consider use of other anti-platelet medications or alternative strategies for Plavix in patients who are poor metabolizers (PM).

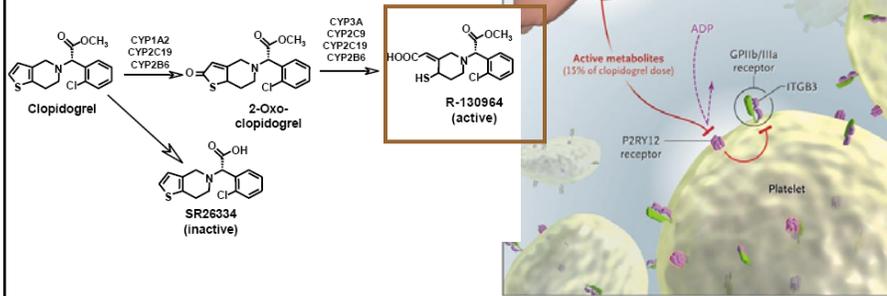
Although a higher dose regimen in PMs or IMs may increase antiplatelet response, an appropriate dose regimen for these patients has not been established in a clinical trial.

- March 12, 2010 - U.S. FDA adds *Boxed Warning* to the label of Plavix.

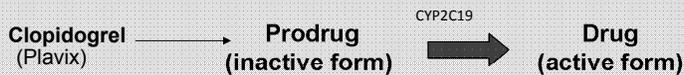
Pharmacogenomics of clopidogrel

Simon, NEJM 2009;360:363-75

Used for the prevention of atherothrombotic events in patients after AMI.



Consequences of Genotype on Patient Response: Prodrug



functional alleles

Clinical phenotype

| | | | |
|----|-----------------|--------------------------|------------------------------|
| 0 | Homo. variant | Poor metabolizer | Risk for ineffective therapy |
| 1 | Hetero. variant | Intermediate metabolizer | |
| 2 | Wild type | Extensive metabolizer | Risk for adverse reaction |
| >2 | Gene dupl | Ultrarapid metabolizer | |

ELEVATE-TIMI 56 TRIAL

- Multicenter, randomized double blinded trial that enrolled patients with cardiovascular disease across 32 sites
- JAMA, 2011, 306: 2221- 2228
- Authors: J. L. Mega et al.

Enrollment Criteria

- Indication for the use of clopidogrel either had MI or PCI > 4 wks but ≤ 6 months
- All patients took a stable aspirin dose daily
- Exclusions
 - Use of anticoagulants
 - Proton pump inhibitors
 - Smoking
 - Prior stent thrombosis
 - Risk of bleeding
 - End stage renal or hepatic disease
 - Planned hospitalization in next 12 weeks

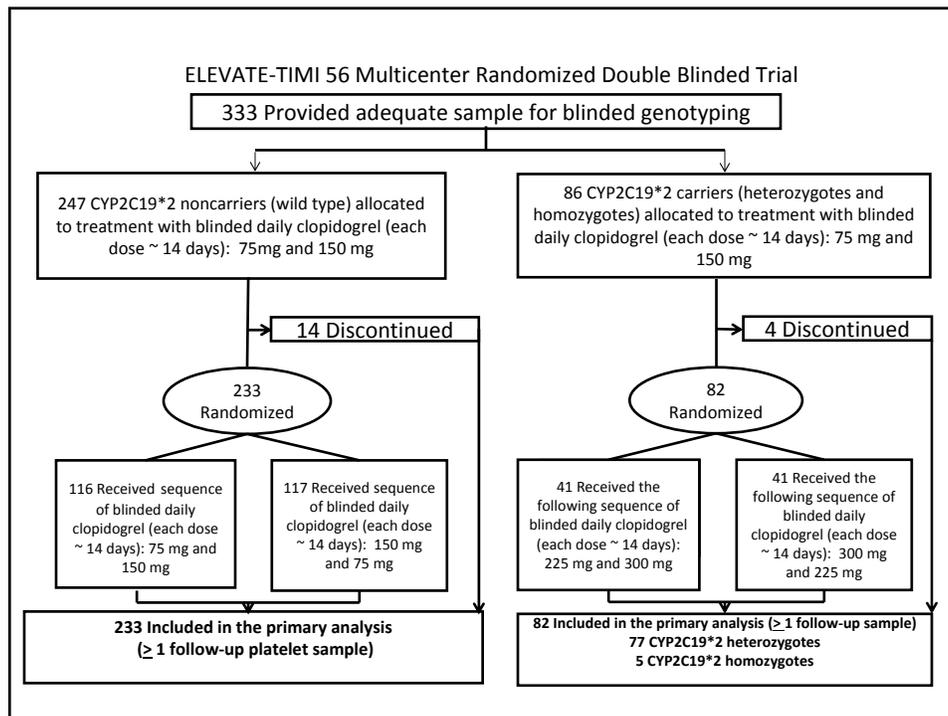
Study Protocol

Baseline blood sampling :

- Genotyping by pyrosequencing
- Platelet function testing by two independent methods

Randomized and blinded sequence of various doses of clopidogrel administered

- At the end of each treatment period of 14 days, platelet function evaluated
- Ischemia, bleeding and other adverse events noted
- Following 1 month completion of study clinical evaluation performed and adverse events recorded



Patients demography

- Total 333 pts underwent genotyping
- Mean age 60 yrs
- 75% male
- 57% MI, 97% PCI
- Clinical characteristics did not differ significantly between carrier and noncarrier (wild type) groups
- **MI: myocardial infarction;**
- **PCI: percutaneous coronary intervention**

Table 1. Patient Baseline Characteristics ^a

| | Overall (N=333) | CYP2C19*2 Noncarriers (n = 247) | CYP2C19*2 Carriers (n = 86) | P Value (Carriers vs Noncarriers) |
|--|--------------------|---------------------------------------|-----------------------------------|---|
| Age, mean, yrs | 60 | 60.8 | 59 | .07 |
| Male sex | 249 | 186 | 63 | .71 |
| Race/ethnicity | | | | .02 |
| White | 293 | 224 | 69 | |
| Black | 30 | 18 | 12 | |
| Asian | 8 | 3 | 5 | |
| Other | 2 | 2 | 0 | |
| Systolic blood pressure, mean (mm Hg) | 126.8 | 126.6 | 127.3 | .67 |
| Diastolic blood pressure, mean (mm Hg) | 76 | 75.4 | 77.5 | .12 |
| Heart rate, mean /min | 68.6 | 68.4 | 69.1 | .38 |
| BMI ^b mean | 76.0 | 75.4 | 77.5 | .12 |
| Hypertension | 287 | 214 | 73 | .68 |
| Hypercholesterolemia | 315 | 232 | 83 | .58 |
| Diabetes Mellitus | 118 | 90 | 28 | .52 |
| Family history of CAD | 137 | 99 | 38 | .51 |
| History of MI | 190 | 134 | 56 | .08 |
| History of PCI | 324 | 240 | 84 | >.99 |
| CABG procedure | 59 | 43 | 16 | .80 |

BMI, body mass index; CABG, coronary artery bypass graft; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention.

^a Unless otherwise indicated, data are presented as No.

^b Calculated as weight in kilograms divided by height in meters squared.

ADP induced platelet aggregation by VerifyNow test



Basically a relative time required to aggregate platelets is measured

rt.org/documents/satellite_programs/ACS/771909/pdf/PRICE_GENEVA_HEART-ORG.pdf - Windows Internet Explorer

www.theheart.org/documents/satellite_programs/ACS/771909/pdf/PRICE_GENEVA_HEART-ORG.pdf

Printes Help

v.theheart.org/documents/satellite_progra...

5 / 19 75%

Principle of the VerifyNow platelet function assay

The diagram illustrates the principle of the VerifyNow platelet function assay. It shows a 'Light source' emitting light through a 'Mixing chamber' containing a grid of beads. Below this, the process is shown in three stages: 1. 'Platelets in whole blood maximally activated by agonist' (represented by blue and green platelets with receptors). 2. 'Fibrinogen-coated beads' (represented by orange beads with fibrinogen molecules). 3. 'Agglutinated beads aggregate in clusters' (represented by the platelets bound to the fibrinogen-coated beads, forming a cluster). The entire process is labeled as 'Agonist'.

Light source

Mixing chamber

Agonist

Platelets in whole blood maximally activated by agonist

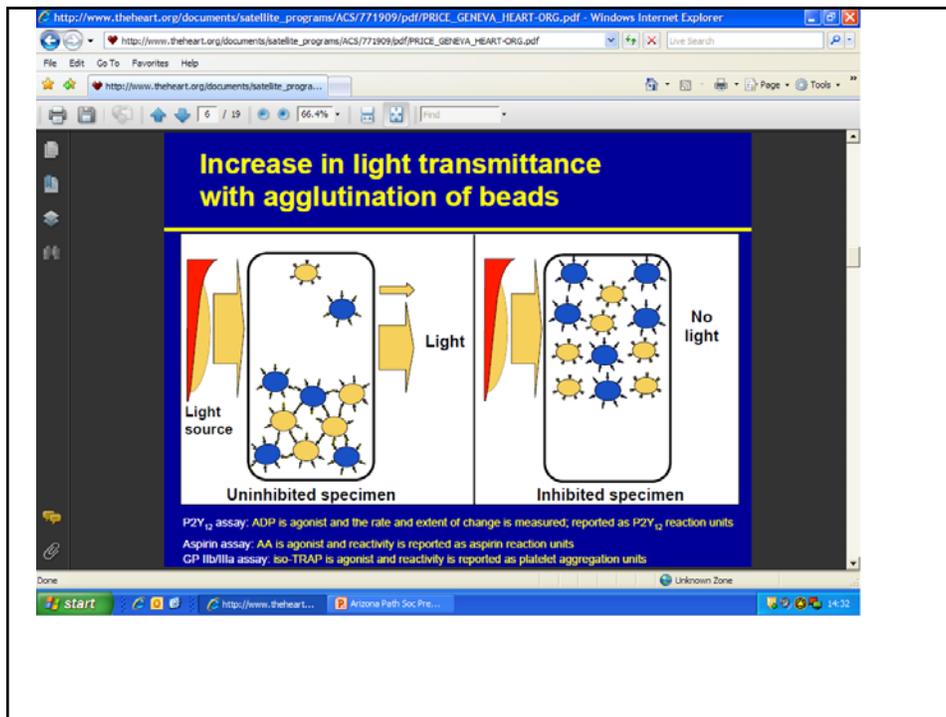
Fibrinogen-coated beads

Agglutinated beads aggregate in clusters

Unknown Zone

http://www.theheart...

14:21



ACCUMETRIC

S VerifyNow P2Y₁₂ Test



- Results given as 3 numbers:
 1. Patient's platelet function with clopidogrel present
 2. Baseline number – 2nd channel activates the platelets independent of P2Y₁₂ receptor with TRAP and PAR4-AP
 3. Percent inhibition of the platelets by clopidogrel
- Less than 20% to 30% inhibition is considered resistant
 - Some people use >235 PRUs as resistant

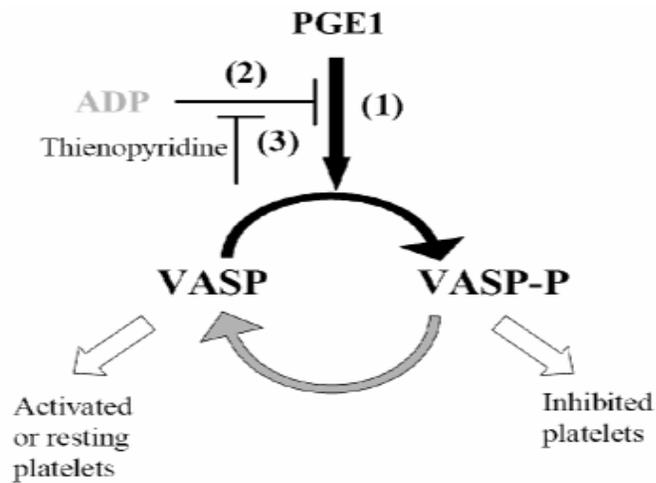
Clopidogrel: Platelet Function Testing

Platelet Reactivity by Flow Cytometry

Platelet Vasodilator Stimulated
Phosphoprotein assessment
(PLT VASP)

by

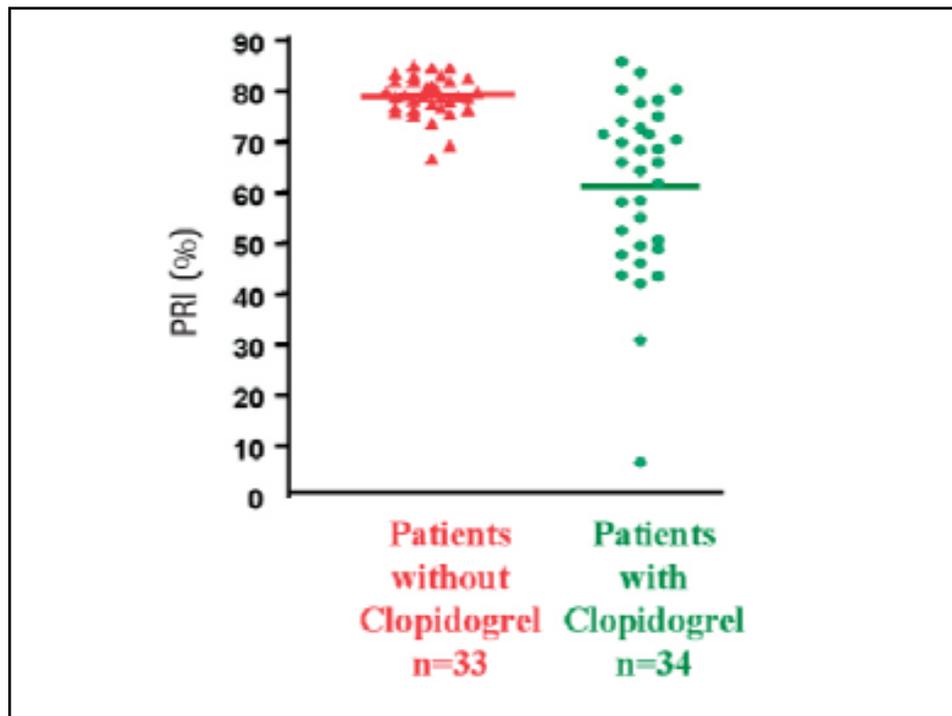
Platelet Reactivity Index (PRI)



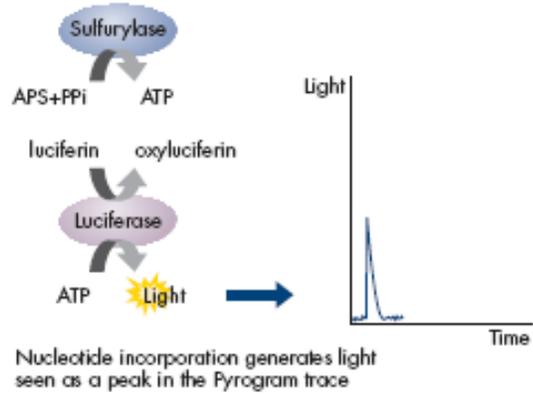
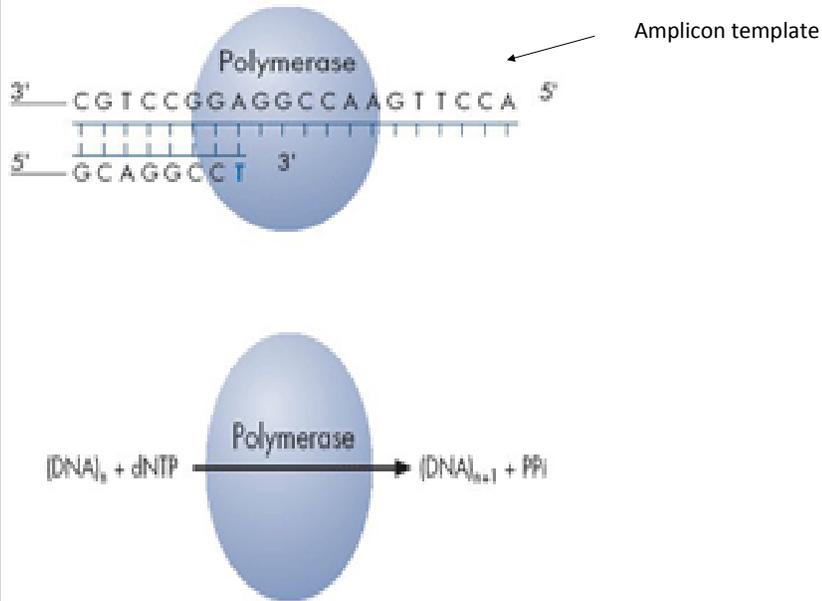
ADP promotes platelet aggregation by acting on P2Y12 receptor. VASP is a platelet protein, non-phosphorylated in basal state. Phosphorylation is activated by a PGE1 mediated pathway in the presence of clopidogrel. VASP phosphorylation inhibits platelet activity even in the presence of ADP.

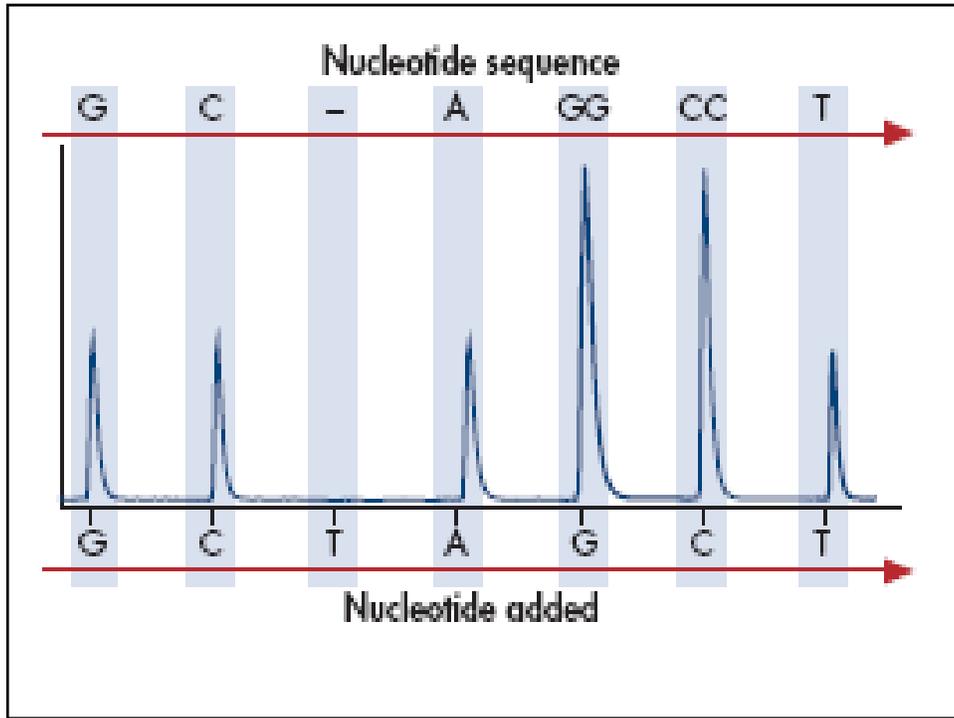
PLT VASP Test

1. Sample + clopidogrel + incubate
2. Sample + clopidogrel + ADP + incubate
3. Following permeabilization the phosphorylated VASP is labeled by immunostaining with a specific mono Ab
4. Platelets are counter-stained by anti CD 61 Ab
5. Dual color flow cytometry provides a platelet reactivity index, PRI (%) from these two conditions



PYROSEQUENCING OF CYP2C19





<http://www.youtube.com/watch?v=nFfgWGF0aA>

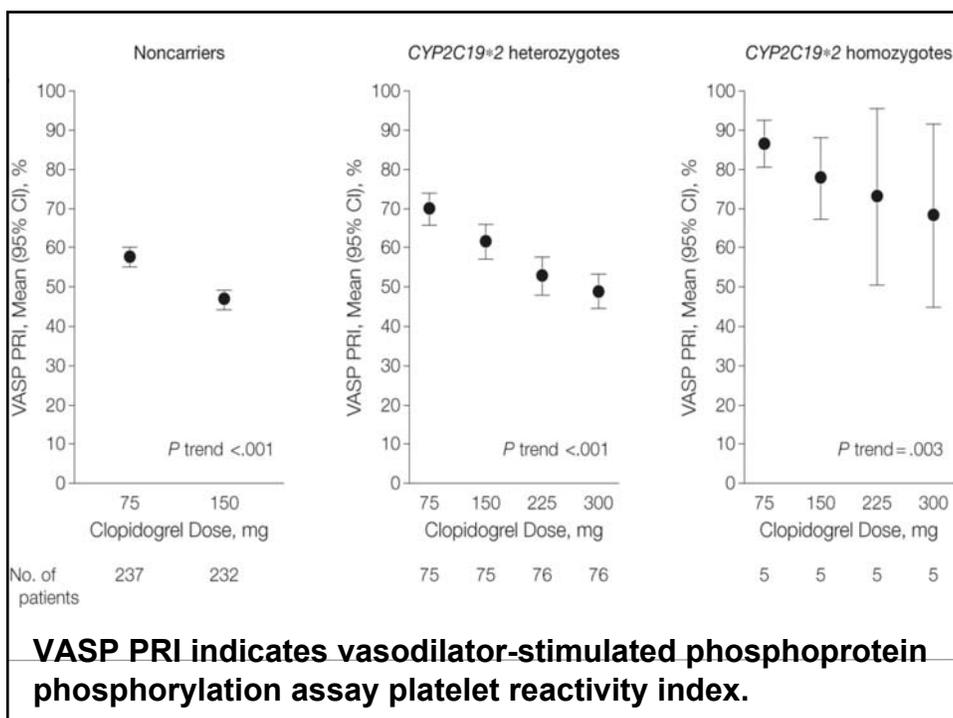
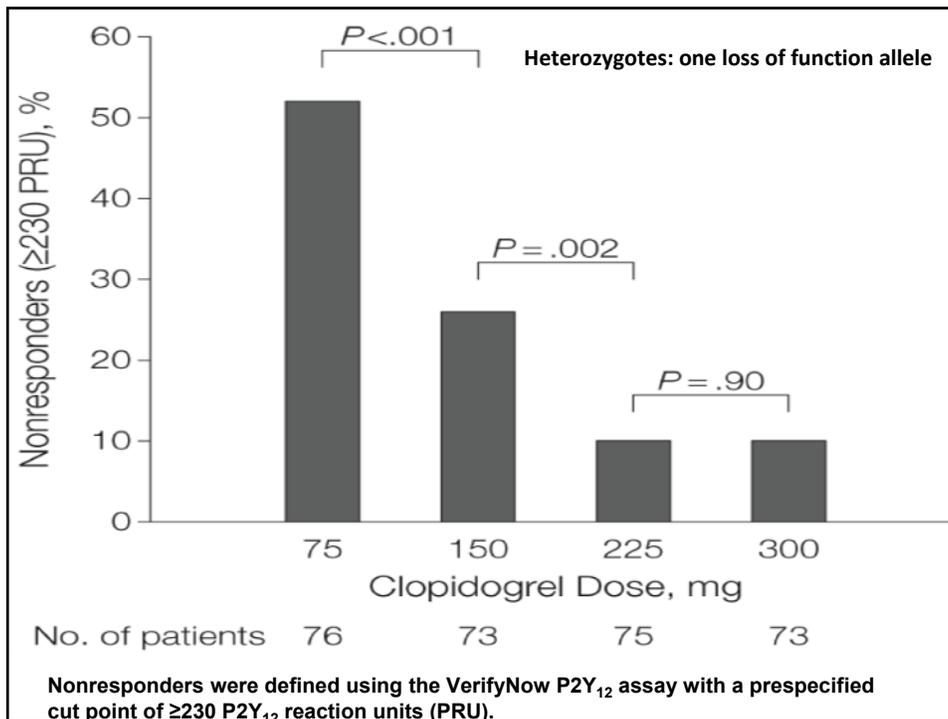


Table 2. On-Treatment Platelet Reactivity

| | VerifyNow PRU | | | | for Trend |
|--------------------------------|--------------------|--------------------|--------------------|--------------------|-----------------|
| | 75 mg | 150 mg | 225 mg | 300 mg | |
| Noncarriers [wild type] | 164 (154-174) | 127 (118-137) | | | <.001 |
| No. of patients | 236 | 230 | | | |
| Carriers | | | | | |
| CYP2C19*2 heterozygotes | 226 (208 - 243) | 188 (171-205) | 153 (135-171) | 128 (110-145) | <.001 |
| No. of patients | 76 | 73 | 75 | 73 | |
| CYP2C19*2 homozygotes | 329 (248 - 410) | 310 (248 - 373) | 286 (178 - 394) | 287 (170 - 404) | 0.32 |
| No. of patients | 5 | 5 | 5 | 5 | |

PRU, platelet reactivity units; 236 had ≥ 1 follow-up platelet function value. 76 heterozygotes and 5 homozygotes also had at least 1 follow-up platelet function value.



Clinical Events

No death, No cerebrovascular events

Noncarriers (WT individual)

- 0 bleeding during 75 mg dose treated group
- 5 bleeding events during 150 mg treat. group

Carriers (heterozygotes for loss of function allele)

- 3 bleeding events in 225 and 300 mg doses

A couple of ischemia events in both groups

Conclusions

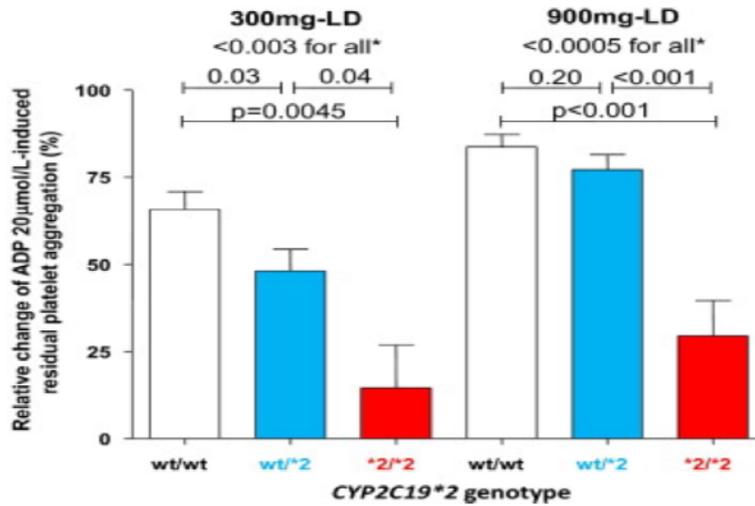
- Tripling clopidogrel (plavix) maintenance dose of 225 mg daily in CYP2C19*2 heterozygote individuals achieved levels of platelet reactivity similar to that seen with the standard 75 mg dose in noncarriers (wild type)
- Even 300 mg daily dose is unlikely to result in optimal degrees of platelet inhibition in homozygote individuals.
 - These patients may be treated with an alternative drug such as prasugrel or ticagrelor both of which do not appear to be affected by CYP2C19 variants

CLOVIS – 2 TRIAL

JACC: Cardiovascular Interventions 2011; 4(4): 392-402

Clopidogrel and Response Variability Study

- MI Patients (109 total) : genotyped for *2 allele
- WT (51), heterozygote (43), homozygote (8)
- Standard loading dose (one group)
- 3 times standard loading dose (2nd group)
 - Follow up : platelet function tests and clinical events
 - Measured active metabolite of clopidogrel by LC:MS/MS



Relative change in ADP- induced RPA. According to CYP2C19*2 and Clopidogrel LDs

Limitations

- Treatment allocation was based on only *2 loss of function alleles. Other polymorphisms of CYP2C19 were not included. However, their prevalence are less than 5%
- Long term tolerability of higher doses require further study



"Here's my
sequence..."