Role of Genotyping and Point-of-Care Testing in Clopidogrel, Prasugrel, and Ticagrelor

Ron Waksman, MD
Professor of Medicine (Cardiology), Georgetown University
Associate Director, Division of Cardiology, Washington Hospital Center
UNDERSTANDING ANTIPLATELET RESPONSE VARIABILITY

Pharmacogenomics, Metabolism, and Platelet Reactivity
Antiplatelet Drug Resistance / Response Variability: An Emerging Clinical Problem

EDITORIAL COMMENT
Aspirin Resistance:
Deepak L. Bhatt, MD, FACC, FSCAI, FESC
Cleveland, Ohio

EDITORIAL COMMENT
Platelet Function Assessment to Predict Outcomes After Coronary Interventions
Fernando Alfonso, MD, PhD, FESC,†
Dominick J. Angiolillo, MD, PhD, FACC‡
Madrid, Spain; and Jacksonville, Florida

Circulation
Clopidogrel Response Variability and Future Therapies
Clopidogrel:
Michelle O’Donoghue, MD; Stephen D. Wiviott, MD
Individual Response Variability to Dual Antiplatelet Therapy in the Steady State Phase of Treatment

Adapted from Angiolillo DJ et al. Am J Cardiol. 2006;97:38-43.
Baseline Platelet Reactivity* Determines Clinical Outcomes** Following Coronary Stenting


* Fibrinogen binding in response to 0.2 µM ADP
** Composite MI, UR, Revascularization
Non-responsiveness to Clopidogrel Is a Predictor of Stent Thrombosis in Patients Receiving a DES

- In 804 patients undergoing stenting, stent thrombosis was found to be more prevalent in patients with post-treatment platelet aggregation $\geq 70\%$ in response to 10 $\mu$M ADP.
- The incidence of stent thrombosis was 8.6\% in nonresponders and 2.3\% in responders ($P<0.001$).

Interindividual Variability in Platelet Reactivity to Clopidogrel in Patients Undergoing Coronary Stenting

2 Hours

24

12

Patients (%)

≤ -30

(-30,-20)

(-20,-10)

(-10,0)

(0,10)

(10,20)

(20,30)

(30,40)

(40,50)

(50,60)

>60

Δ Aggregation (%)

20

10


24 Hours

20

10

Patients (%)

≤ -30

(-30,-20)

(-20,-10)

(-10,0)

(0,10)

(10,20)

(20,30)

(30,40)

(40,50)

(50,60)

>60

Δ Aggregation (%)

5 Days

22

11

Patients (%)

≤ -10

(-10,0)

(0,10)

(10,20)

(20,30)

(30,40)

(40,50)

(50,60)

>60

Δ Aggregation (%)

30 Days

28

14

Patients (%)

≤ -10

(-10,0)

(0,10)

(10,20)

(20,30)

(30,40)

(40,50)

(50,60)

>60

Δ Aggregation (%)

Platelet Function Tests

- Platelet Aggregation
  - Impedance platelet aggregation
  
- Flow Cytometry
  - GPIIb/IIIa receptor activation
  - P-selectin expression
  - Monocyte-platelet aggregates
  - Vasodilator-associated stimulated phosphoprotein (VASP)

- Point-of-care
  - Ultegra rapid platelet function analyzer (VerifyNow)
  - Thromboelastagraph (TEG)
  - PFA-100
  - Plateletworks
  - Cone and plate(let) analyzer (IMPACT)

- Genetic testing
How does the VerifyNow Assay Work?

- Whole blood, closed-tube sampling with no pipetting required
- Assay results in less than 5 minutes (assay time)
- Good correlation with LTA and VASP

Diagram:

1. Open the cover
2. When prompted, insert the assay device until it clicks.
3. When prompted, insert the tube onto the assay device until it clicks.
4. Close the cover after inserting the tube and read results in 2 to 5 minutes.

Agonists:
- Aspirin Assay – AA
- P2Y12 assay – ADP + PGE1
- GpIIbIIa assay – iso-TRAP

Light Source

Mixing Chamber

Agonist

Platelets in whole blood maximally activated by agonist in mixing chamber

Fibrinogen-coated beads

Agglutinated beads aggregate in clusters
The GRAVITAS Trial Design

Gauging Responsiveness with a VerifyNow Assay – Impact on Thrombosis and Safety

- This trial is designed to evaluate whether tailored clopidogrel therapy, using a point-of-care platelet function assay, reduces major adverse cardiovascular events after DES implantation.

Stable angina/ischemia or non-ST-elevation acute coronary syndrome undergoing PCI with DES

Not High Residual Platelet Reactivity on Clopidogrel Therapy 12 to 24 hours Post PCI

- Standard Dosing
  - Clopidogrel 75 mg once daily x 6 months

High Residual Platelet Reactivity on Clopidogrel Therapy 12 to 24 hours Post PCI

- High Dose
  - Clopidogrel 450 mg loading dose followed by 150 mg once daily x 6 months

- The primary end point is the time to first occurrence of cardiovascular death, nonfatal myocardial infarction, or definite/probable stent thrombosis.

Power Analysis: Sample Size Estimate

• Assumptions:
  • An event rate of 5% in patients on standard-dose clopidogrel at 6-months
  • 50% risk reduction with high-dose clopidogrel
  • 2200 patients needed to provide 80% power at a two-sided 0.05 significance level
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)

Clopidogrel High Dose N=1109

Clopidogrel Standard Dose N=1105
Primary Endpoint: CV Death, MI, Stent Thrombosis

2.3% vs. 2.3%
HR 1.01 (95% CI 0.58 - 1.76)
p=0.98

Observed event rates are listed; P value by log rank test.
Bleeding Events: Safety Population

Severe or life-threatening: Fatal bleeding, intracranial hemorrhage, or bleeding that causes hemodynamic compromise requiring blood or fluid replacement, inotropic support, or surgical intervention.

Moderate: Bleeding that leads to transfusion but does not meet criteria for severe bleeding.

P by log rank test; observed event rates listed. HD, high-dose; SD, standard dose.
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
## Baseline Characteristics: Non-Randomized Comparison

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SD – High RPR N=1105</th>
<th>SD – Not High RPR N=586</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual platelet reactivity, median (IQR)</td>
<td>283 PRU (255 - 321)</td>
<td>151 PRU (105 - 191)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, years</td>
<td>64 ± 11</td>
<td>62± 10</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Male sex</td>
<td>65%</td>
<td>80%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>47%</td>
<td>29%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (median)</td>
<td>31</td>
<td>29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cr Cl&lt; 60 ml/min</td>
<td>42%</td>
<td>27%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>30%</td>
<td>20%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Indication for PCI</td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>Stable angina or ischemia</td>
<td>60%</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>UA, no ST depression</td>
<td>24%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>NSTE-ACS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA, ST-dep, biomarker (-)</td>
<td>5%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Cardiac biomarker (+)</td>
<td>10%</td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>
Secondary Comparison: High vs. Not High Reactivity

Observed event rates are listed. P value by log-rank test.
CV Events and Post-PCI PRU In Pts With High and Not High Reactivity Treated With Standard-Dose Clopidogrel

PRU 12 - 24 hrs post-PCI

Red dots: patients with CV death, MI, or ST

High Residual Reactivity

N=1105

Not High Residual Reactivity

N=586

ITT population
**GRAVITAS: Possible Explanations**

- Underpowered: patients low-risk, low event rates.
  - Given HR of 1.01 after 2200 patients, unlikely that a larger trial would show a clinically meaningful benefit
- Pharmacodynamic effect of the intervention was too weak?
  - Stronger intervention, goal-directed therapy with serial measurements merit study
- Platelet reactivity is a non-modifiable risk factor?
  - To be further examined in TARGET-PCI, ARCTIC, TRIGGER-PCI
- VerifyNow results not predictive of risk?
  - However, at least 7 studies involving more than 3,000 patients demonstrate a correlation with MACE
GRAVITAS does not support a treatment strategy of high-dose clopidogrel in low-risk patients with high reactivity identified by a single platelet function test after PCI.
UNDERSTANDING PLATELET GENOMICS

Pharmacogenomics, Metabolism, and Platelet Reactivity
Factors Affecting Response to Clopidogrel

Compliance

Absorption
- MDR1
- ... 

Drug interaction
- CYP2C19
- CYP2C9
- CYP2B6
- CYP3A4
- CYP3A5
- CYP1A2

Uptake/Elimination

Bioactivation

Drug interaction

Platelet turnover

Signal transduction

Receptor
- P2Y12
- GP IIb/IIIa

ACS, diabetes, age, BMI...
Mechanism of Action of Prasugrel

**Ticagrelor: Pharmacology**

- A P2Y$_{12}$ purinoceptor antagonist
- Does not require cytochrome P450 metabolic activation to exert its inhibitory effects on platelet aggregation
- No active metabolite
- Rapid onset of action
- Reversibly binds to the P2Y$_{12}$ receptor
  - Potential advantage if needing to discontinue therapy due to surgery
- Compared with clopidogrel, produces a greater and more consistent inhibition of ADP-induced platelet aggregation

Effects of CYP2C19*2 and *17 Combined

N=445 patients; 24 hours post 600-mg clopidogrel loading dose

CYP2C19 and CVD, MI, or Stroke

N=1477 ACS/PCI Subjects Treated with Clopidgrel in TRITON-TIMI 38

- CYP2C19 Reduced-Function Allele Carriers
  - Hazard Ratio 3.09 (95% CI 1.19-8.00)
  - P=0.015

- Non-carriers
  - Hazard Ratio 1.53 (95% CI 1.07-2.19)
  - P=0.014

* Carriers ~30% of the population

CYP2C19*2 and Outcomes

Stent Thrombosis:
(HR 6.02, 95% CI 1.81-20.04, P=0.0009)

CYP2C19*2 and Outcomes

P=0.003

# CYP2C19 and Treatment with Clopidogrel

## Predominantly for PCI

### CVD, MI, or Stroke

<table>
<thead>
<tr>
<th></th>
<th>N=9,685</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or 2 CYP2C19 RFA vs Non-Carriers</td>
<td>91.5% PCI</td>
<td>1.57 (1.13-2.16)</td>
<td>0.006</td>
</tr>
<tr>
<td>1 CYP2C19 RFA vs Non-Carriers</td>
<td></td>
<td>1.55 (1.11-2.17)</td>
<td>0.01</td>
</tr>
<tr>
<td>2 CYP2C19 RFA vs Non-Carriers</td>
<td></td>
<td>1.76 (1.24-2.50)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### Stent Thrombosis

<table>
<thead>
<tr>
<th></th>
<th>N=5,894</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or 2 CYP2C19 RFA vs Non-carriers</td>
<td></td>
<td>2.81 (1.81-4.37)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1 CYP2C19 RFA vs Non-Carriers</td>
<td></td>
<td>2.67 (1.69-4.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 CYP2C19 RFA vs Non-Carriers</td>
<td></td>
<td>3.97 (1.75-9.02)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*RFA=reduced-function allele

CURE Genetics Substudy

No association seen between \textit{CYP2C19} and outcomes in clopidogrel arm.

\textbf{BUT}, patients treated conservatively (only \(~15\%) rate of PCI).

Magnitude of PGx Interaction Will Depend on Relative Benefit of Clopidogrel

Conservatively managed

Invasively managed

20% Risk Reduction

Aspirin Monotherapy

85% Risk Reduction

CURE. NEJM 2001;345:494-502

CYP2C19 and Treatment with Clopidogrel

CVD, MI, or Stroke: Carriers of 1 or 2 CYP2C19 Variants vs Non-Carriers

<table>
<thead>
<tr>
<th>Study</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meta-Analysis (91.5% PCI)</td>
<td>1.57 (1.13-2.16)</td>
</tr>
<tr>
<td>PLATO (66% planned PCI)</td>
<td>1.43 (1.11-1.84)</td>
</tr>
<tr>
<td>Meta-Analysis + PLATO</td>
<td>1.43 (1.11-1.84)</td>
</tr>
<tr>
<td>CURE (15.5% PCI)</td>
<td></td>
</tr>
<tr>
<td>CHARISMA (stable CAD or risk factors)</td>
<td>1.32 (1.07-1.63)</td>
</tr>
</tbody>
</table>

Risk Higher with CYP2C19 Variant

Risk Lower with CYP2C19 Variant

Alternative Treatments: Pharmacodynamics

Clopidogrel Responder
Clopidogrel Non-responder
*Responder = ≥25% IPA at 4 and 24 h

Brandt JT et al., Am Heart J 2007;153:e9-e16.

TRITON-TIMI 38 Genetic Substudy

1477 Pts w/ ACS and Planned PCI
Clopidogrel

1466 Pts w/ ACS and Planned PCI
Prasugrel

P=0.046 for interaction between benefit of prasugrel vs. clopidogrel and CYP2C19 genotype

CYP2C19 and Outcomes:
Clopidogrel and Ticagrelor, N=10,285

CV Death, MI, Stroke

Days from randomization

K-M estimate (%)

No. at risk
Clopidogrel LOF 1,388 1,275 1,259 1,226 1,027 801 658
Clopidogrel No LOF 3,516 3,321 3,256 3,186 2,691 2,123 1,757
Ticagrelor LOF 1,384 1,305 1,274 1,250 1,053 834 683
Ticagrelor No LOF 3,554 3,352 3,301 3,222 2,718 2,127 1,761

Wallentin et al. Lancet. 2010; Online, 2010 DOI:10.1016/S0140-6736(10)61274-3
FDA Drug Safety Communication: Reduced effectiveness of Plavix (clopidogrel) in patients who are poor metabolizers of the drug

Safety Announcement
Additional Information for Patients
Additional Information for Healthcare Professionals
Data Summary

Safety Announcement
[03-12-2010] The U.S. Food and Drug Administration (FDA) has added a Boxed Warning to the label for Plavix, the anti-blood clotting medication. The Boxed Warning is about patients who do not effectively metabolize the drug (i.e. "poor metabolizers") and therefore may not receive the full benefits of the drug.

The Boxed Warning in the drug label will include information to:
- Warn about reduced effectiveness in patients who are poor metabolizers of Plavix. Poor metabolizers do not effectively convert Plavix to its active form in the body.
- Inform healthcare professionals that tests are available to identify genetic differences in CYP2C19 function.
- Advise healthcare professionals to consider use of other anti-platelet medications or alternative dosing strategies for Plavix in patients identified as poor metabolizers.

Plavix is given to reduce the risk of heart attack, unstable angina, stroke, and cardiovascular death in patients with cardiovascular disease. Plavix works by decreasing the activity of blood cells called platelets, making platelets less likely to form blood clots.

For Plavix to work, enzymes in the liver (particularly CYP2C19) must convert (metabolize) the drug to its active form. Patients who are poor metabolizers of the drug do not effectively
Collaborative Meta-analysis: CYP2C19 and Stent Thrombosis in Patients on Clopidogrel

Risk Ratio (95% CI)  P value

Carriers vs Noncarriers  2.81 (1.81-4.37)  < .0001
Heterozygotes vs Wild Type  2.67 (1.69-4.22)  < .0001
Homozygotes vs Wild Type  3.97 (1.75-9.02)  < .001

N = 5772

Risk Lower With CYP2C19 Variant
Risk Higher With CYP2C19 Variant

Mega JL. American Heart Association; November 2009; Orlando, Florida.
Pharmacogenomics of Antiplatelet Therapies

Carriers vs Non-carriers of a Reduced-function CYP2C19 Allele

CLINICAL OUTCOMES

<table>
<thead>
<tr>
<th>Drug</th>
<th>Odds Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prasugrel</td>
<td>0.89</td>
<td>0.27</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>1.53</td>
<td>0.01</td>
</tr>
</tbody>
</table>


Event-free Survival Over 1 Year of Follow-up

* In Patients Treated with Clopidogrel Following PCI*

No. of CYP2C19*2 alleles

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
</tr>
</thead>
</table>

CYP2C19*2 variant accounts for 12% clopidogrel response variation of platelet aggregation to ADP.

TRITON-TIMI 38

ABCB1 and CYP2C19 Polymorphisms Diminish Clopidogrel Antiplatelet Effect

Both polymorphisms = highest risk

No polymorphisms = lowest risk

p=0.0018 across genotype.

CC = ABCB1 normal function; C→T = ABCB1 reduced function; Post-hoc analysis.

Genetic and Platelet Function Testing

GENOME
- ~2x10^4 genes
- ~10^7 SNPs
- ~10^5 transcripts

TRANSCRIPTOME
- >10^5 proteins

PROTEOME
- ~10^5 proteins
- ~10^6 modified proteins

ENVIRONMENT

PHENOTYPE

PLATELET FUNCTION TESTING
- Proximal to phenotype
- Captures environmental & genetic variability
- More difficult to assess
- Varies with time

GENETIC TESTING
- Fixed
- Lifelong impact
- Easy to assess
- Distanced from phenotype

Translation
Post-translation modification

Distances from phenotype

Captures environmental & genetic variability

More difficult to assess
Varies with time
Clopidogrel Reloading Among Genetic Carriers

Bonello et al JACC 2010
Tailoring Antiplatelet Therapy Based on Platelet Function Testing and Genotyping

- Paul Gurbel, August 2010
  "The bottom line is we have no prospective studies at this time that alteration of therapy based on genotype or phenotype really affects patient outcomes"

- White Paper, JACC 2010 (Bonello/Gurbel et al)
  “However, until the results of large scale trials of personalized antiplatelet therapy are available, the routine use of platelet function measurements in the care of patients with cardiovascular disease cannot be recommended”

- 2010 ACCF/ACG/AHA Expert Consensus Document on the Concomitant Use of PPIs and Thienopyridines
  “The role of either pharmacogenomic testing or platelet-function testing in managing therapy with thienopyridines and PPIs has not yet been established”
Conclusions

• The CYP2C19 reduced function genotypes are associated with worse outcomes in the setting of treatment with clopidogrel. Novel antiplatelets appear to be less so.

• ABCB1 and PON-1 may offer similar risk stratification.

• Genetics and platelet function appear to offer complementary information.

• Routine use of genetic and platelet function testing for alteration of therapy is not ready for prime time.