

## CHAPTER

# 13

## *Resistance to Antiplatelet Drugs- Current Perspective & Future Directions*

**H. M. Mardikar, D. Deo, A. Ghosh,  
N. V. Deshpande**

Platelets play a key role in the pathophysiology of thrombosis after plaque rupture.<sup>1</sup> Plaque rupture occurs spontaneously in patients with acute coronary syndromes (ACS) or may be iatrogenically induced in patients undergoing percutaneous coronary interventions (PCI). The rationale for a review article on resistance to antiplatelet drugs in patients with coronary artery disease (CAD) is the current intense controversy surrounding the clinical importance of non-responsiveness to antiplatelet therapy.<sup>2-6</sup>

### **Clopidogrel Resistance**

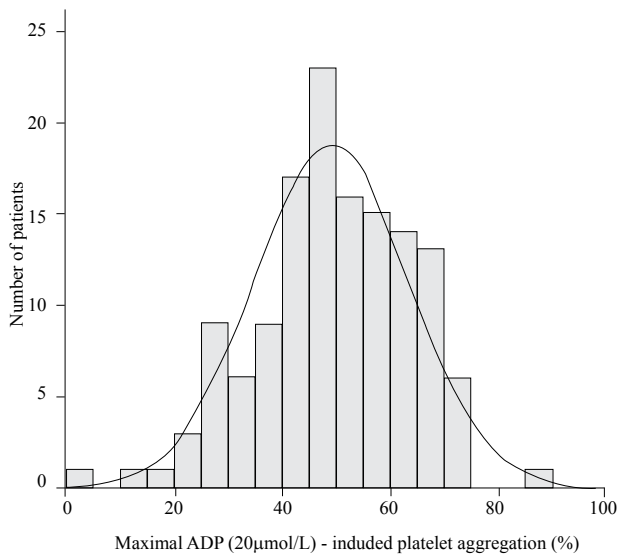
There are multiple mediators of platelet activation. One of the chief mediators is adenosine diphosphate (ADP) which binds to the P2Y<sub>12</sub> receptor on platelets.<sup>7</sup> Thienopyridines are irreversible inhibitors of the ADP/P2Y<sub>12</sub> receptor. Ticlopidine, a first-generation thienopyridine in combination with aspirin has been shown to be beneficial and superior to oral anticoagulants in preventing thrombotic complications after PCI with Stenting.<sup>8-11</sup> Clopidogrel is a second-generation thienopyridine with similar efficacy and has largely replaced ticlopidine due to its better tolerability profiles.<sup>12</sup> Combination of Clopidogrel & Aspirin is currently the antiplatelet treatment of choice for prevention of stent thrombosis.<sup>13</sup> In addition, in patients undergoing PCI, prolonged dual antiplatelet therapy

has been associated with better long-term clinical outcomes.<sup>14,15</sup> The CURE Trial has shown clinical benefit with dual antiplatelet therapy in patients with unstable angina and non-ST-segment elevation myocardial infarction (NSTEMI) independent of coronary revascularization.<sup>16</sup> Recently, the clinical benefit of clopidogrel has also been observed in patients with ST-segment elevation myocardial infarction (STEMI).<sup>17,18</sup>

Variable response to antiplatelet therapy is well known and it is quite well documented with clopidogrel. From the clinical point of view, the patients who suffer acute coronary event on regular clopidogrel therapy can be called as clopidogrel resistant. Recent studies have suggested a relation between high post-treatment platelet reactivity and clopidogrel nonresponsiveness in patients undergoing percutaneous revascularization and increased ischemic events including stent thrombosis.<sup>19,20</sup> Variable responsiveness to clopidogrel has served as a rationale for the development of new P2Y<sub>12</sub> inhibitors with superior pharmacodynamic profiles.<sup>21</sup>

### **Mechanism of action of Clopidogrel**

Clopidogrel selectively and irreversibly inhibits the P2Y<sub>12</sub> receptor on the platelets.<sup>22</sup> Clopidogrel is an inactive pro-drug that requires oxidation by the hepatic cytochrome P450 (CYP) system to generate

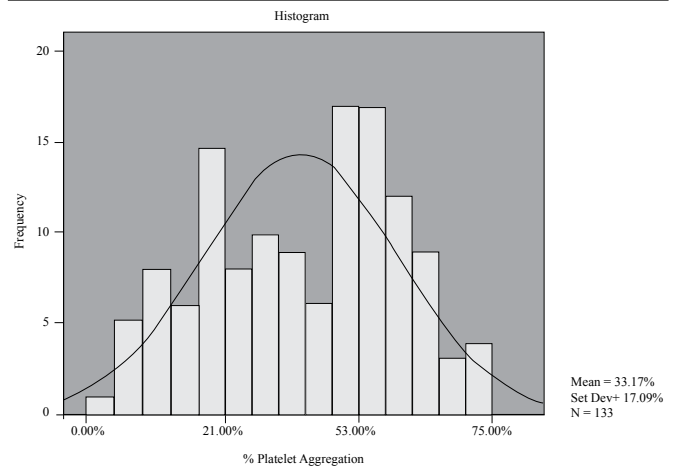
**Figure 1a: Interindividual Variability in Platelet Aggregation in Western patients**

Platelet aggregation profile (maximal 20 µmol/l ADP-induced platelet aggregation using light transmittance aggregometry) in patients (n = 135) in a steady state phase (> 1 month) of combined aspirin (100 mg/day) and clopidogrel (75 mg/day) therapy. Heterogeneous antiplatelet effects are observed in the overall patient population as depicted by the normal bell-shaped distribution of platelet aggregation. ADP = adenosine diphosphate (D.J. Angiolillo, unpublished data, 2006).

an active metabolite. However, approximately 85% of the pro-drug is hydrolyzed by esterases in the blood to an inactive carboxylic acid derivative, and the remaining is metabolized by the CYP system in the liver to generate an active metabolite.

### Definition of Clopidogrel Responsiveness

Though variable response to clopidogrel is well documented, standardized definitions to define individual responsiveness to clopidogrel are still lacking. The reason for this is multi-factorial. There are numerous assays to assess clopidogrel-induced antiplatelet effects. There is methodological variability within each technic. The same group of researchers has used different concentration of platelet agonists in their studies. This issue is further confused by different terminology used like “low-responder,” “hypo-responder,” “semi-responder” and “suboptimal responder”. Added

**Figure 1b : Interindividual Variability in Platelet Aggregation in Indian patients**

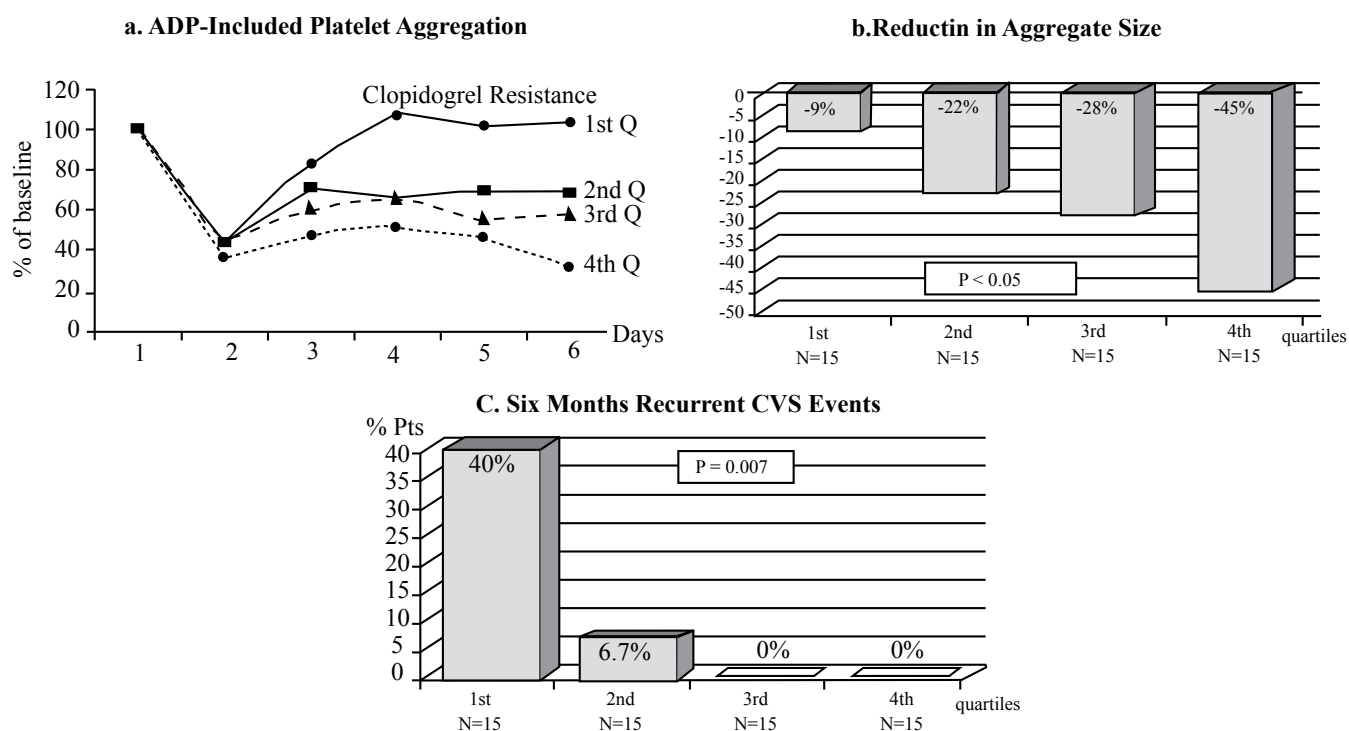
Platelet aggregation was assessed by chronolog lumi-aggregometer model 490-2D. Response was expressed as % platelet aggregation after stimulation with 10 µmol/L ADP. significant variability in the response to clopidogrel among Asian Indians patients with recent ACS or PCI with at least a quarter of patients showing inadequate response to inhibition of platelet aggregation. H. Mardikar et al. Variability in clopidogrel response among Asian Indians unpublished data.

to this is the lack of standardized values to define resistance.

In view of above facts clopidogrel responsiveness should be considered as a continuous and variable parameter. Figure 1a shows variable response to clopidogrel in western population<sup>23</sup> and Figure 2b shows variable response in Indian population (Study done at Spandan Heart Institute & Research Center, Nagpur).

### Clinical implications of variability in response to clopidogrel

There are many studies which have correlated individual response variability to clopidogrel with clinical outcomes. Clinical outcomes include stent thrombosis, post-stent ischemic events and periprocedural myocardial infarction. These studies have been performed in different subgroups of patients undergoing elective PCI for ST elevation MI and non-ST-segment elevation (NSTE)-ACS.

**Figure 2**

Study patients (pts) were stratified into quartiles according to degree of platelet activity inhibition in response to clopidogrel treatment. Patients in 4 quartiles were compared with regard to (a) changes in ADP-induced platelet aggregation expressed as percentage of baseline activity; (b) percentage reduction in aggregate size at day 6 compared with baseline values; and (c) incidence of recurrent major adverse cardiovascular events during a 6-month follow-up.<sup>26</sup>

### Stent thrombosis

The first landmark study to hypothesize the clinical implications of clopidogrel responsiveness was reported by Muller et al.<sup>24</sup> In this study, 105 patients undergoing PCI were enrolled and 2 patients had sub-acute stent thrombosis. Another important study to document this was the CREST (Clopidogrel Effect on Platelet Reactivity in Patients With Stent Thrombosis) study. This study showed that high post-treatment platelet reactivity and incomplete P2Y<sub>12</sub> were risk factors for subacute stent thrombosis.<sup>25</sup>

**Studies in STEMI and NSTEMI:** Matetzky et al.<sup>26</sup> presented the first study suggesting the impact of clopidogrel-induced antiplatelet effects on post-stent ischemic events in patients of ST elevation MI following PCI. In this study, 60 Patients were stratified into 4 quartiles according to the percentage reduction of ADP-induced platelet

aggregation. Whereas 40% of patients in the first quartile sustained a recurrent cardiovascular event (STEMI, ACS, subacute stent thrombosis, and acute peripheral arterial occlusion) during 6-month follow-up, only 1 patient (6.7%) in the second quartile and none in the third and fourth quartiles suffered a cardiovascular event ( $p = 0.007$ ).

Similar study was performed on Non ST Elevation MI by Cuissier et al.<sup>27</sup> studied a series of 106 NSTEMI-ACS patients treated with PCI.

### How to Overcome Poor Clopidogrel Response<sup>28</sup>

Inadequate antiplatelet effect of clopidogrel resistance is a fast emerging clinical entity with potentially severe consequences.<sup>29</sup> Therefore, how to overcome poor clopidogrel response becomes a therapeutic challenge to every physician.

## A. Detection

The initial approach has to be identifying patients who have inadequate response to clopidogrel therapy. There are various methods which are listed below to assess response to antiplatelet therapy (Aspirin, Clopidogrel or GP IIb /IIIa Inhibitors). Full review of this is beyond the scope of the article and interested reader can refer to the listed references.

## Methods of Measuring Platelet function

### Platelet Aggregation Assays

The historical “gold standard” test is light transmittance aggregometry (turbidimetric) (LTA), which is based on the stimulation of platelet-platelet aggregation in platelet-rich plasma after stimulation with various agonists. Light transmittance aggregometry has been the most widely used technique to monitor the effects of antiplatelet agents, including aspirin, clopidogrel, other P2Y<sub>12</sub> inhibitors and platelet glycoprotein (GP) IIb/IIIa inhibitors.

### Receptor Expression

The resting and stimulated expression of activation-dependent receptors can be quantified by flow cytometry with monoclonal antibodies. The most widely studied receptors include P-selectin and GP IIb/IIIa.

### Intracellular

The coupling of P2Y<sub>12</sub> to the inhibition of adenylate cyclase by an inhibitory G protein has been exploited to measure reactivity of the receptor in the presence of P2Y<sub>12</sub> inhibitors.

### Point of Care Assays

- a. **Verify Now** : The Verify Now (Accumetrics, San Diego, California) method uses arachidonic acid, adenosine diphosphate (ADP), or thrombin receptor-activating peptide (TRAP) to assess platelet responsiveness to aspirin, P2Y<sub>12</sub> inhibitors, or GP IIb/IIIa inhibitors, respectively

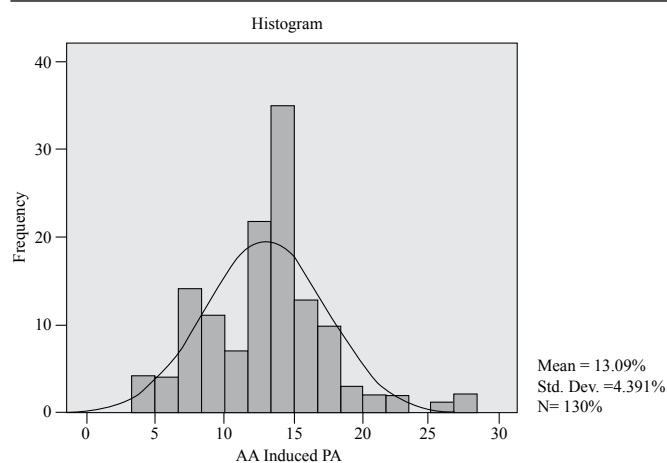
- b. **Thromboelastogram (TEG)** : In the thromboelastogram (TEG) PlateletMapping technic (Hemoscope Corporation, Niles, Illinois), the contribution of arachidonic acid-induced platelet aggregation and ADP-induced aggregation to the overall tensile strength of a platelet–fibrin clot can be quantified and correlated with turbidimetric aggregometry.

Both these assays are most suitable in clinical practice but are expensive. Per patient cost for studying Aspirin or Clopidogrel Resistance varies from Rs. 1600 to Rs. 3000.

## B. Treatment

Several studies have documented importance of adequate dose of loading prior to PCI. The CLEAR PLATELETS (Clopidogrel Loading with Eptifibatide to Arrest PLATELET reactivity) and CLEAR PLATELETS Ib studies showed that in patients (n = 120) undergoing elective PCI, a 600-mg loading dose of clopidogrel was associated with superior early platelet inhibition compared with a 300 mg loading dose.<sup>30,31</sup> This enhanced platelet inhibition was sustained over 24 h and was accompanied by a decrease in release of myocardial necrosis and inflammatory markers. This study clearly documented importance of loading with 600 mg clopidogrel vs. 300 mg of clopidogrel. Recently published one more study has shown advantage of higher dose of clopidogrel loading in patients of ACS.<sup>32</sup>

Current maintenance dose of Clopidogrel is 75 mg/day. This dose was chosen because the degree of platelet inhibition is similar to that achieved by Ticlopidine 25 mg BID.<sup>33</sup> The OPTIMUS (Optimizing anti-Platelet Therapy In diabetes Mellitus) study selectively studied diabetes mellitus patients with high post-treatment platelet reactivity while in their chronic phase of treatment.<sup>34</sup> In these patients, although a 150 mg clopidogrel maintenance dose resulted in marked platelet inhibition of

**Figure 3**

130 patients were studied. The response of subjects to aspirin followed a normal, bell shaped distribution curve with a mean and standard deviation (S.D.) of  $13.1 \pm 4.4\%$  (figure). 3.1% patients had PA values more than 2 S.D. of the mean, hence termed as hypo-responders to aspirin while another 3.1% patients had PA values less than 2 S.D. of the mean, hence termed as hyper-responders to aspirin.

numerous platelet function measures compared with a 75 mg dose, a considerable number of patients still remained above the therapeutic threshold of post-treatment platelet reactivity used in this study, suggesting the need for more potent P2Y<sub>12</sub> inhibitors or alternative antithrombotic regimens in these high-risk patients.

The ongoing CURRENT/OASIS-7 (Clopidogrel optimal loading dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for InterventionS) trial will evaluate whether high-dose clopidogrel achieves better clinical outcome than standard dose in ~ 14,000 NSTEMI-ACS patients undergoing PCI.

## Aspirin Resistance

Aspirin resistance has been a common topic of research and discussion for a while now. From a clinician's perspective, recurrent events in patients of aspirin therapy can be termed as aspirin resistance. The prevalence of aspirin resistance varied from 5- 40 % depending on criteria and methods used to define aspirin resistance. A recently conducted

study has shown 0.5% prevalence of aspirin resistance by thromboelastography<sup>36</sup> and this is not correlated with clinical events. Very similar to clopidogrel resistance, this clinical entity is also confusing because of different methods used to document aspirin resistance. With LTA method and using AA and ADP as agonists, Gum et al<sup>36</sup> reported 5% prevalence of aspirin resistance in patients with stable Coronary Artery Disease. With Ultegra Point-of-care assay this prevalence varies from 19-23%. Another point of care system PFA-100 (Dade-Behring Inc., West Sacramento, California) point-of-service assay, which uses collagen and epinephrine as the agonists, the prevalence of aspirin resistance was 35% in post-myocardial infarction patients.<sup>37</sup>

The detailed discussion on Aspirin Resistance will consume similar space as clopidogrel resistance and hence not duplicated here. Its worth mentioning that real life incidence of aspirin or clopidogrel resistance is not very well documented. Whether this varies in different population is not known. In our study done at Spandan Heart Institute, Nagpur<sup>38</sup> (Figure 3), there were 2.5 % patients resistance to aspirin when LTA was used and AA was used as the agonist. As compared to GUM et al this is less by 50%.

One more factor which one must consider while studying aspirin resistance is aspirin compliance. Due to severe gastric irritation there may be less compliance than expected. In our study<sup>38</sup>, we only enrolled the patients who confirmed that they were taking aspirin regularly. Close to 10 % of patients who were supposed to be enrolled in this study had to be excluded because of non-compliance. The interesting fact is many patients understand that aspirin or aspirin containing pill (polypill) is the cause for gastric disturbance and themselves resort to intermittent therapy of aspirin as well. This fact is also highlighted in the study of Gurbel et al and one must be careful in evaluating aspirin resistance and exclude patients of pseudo aspirin resistance due to non-compliance.



## Future Directions

The current therapeutic alternative for treatment of patients with poor clopidogrel response remains limited. Novel P2Y<sub>12</sub> receptor antagonists with more potent antiplatelet effects are currently under clinical investigation.<sup>35</sup> These novel molecules are all characterized by more potent antiplatelet effects, reduced interindividual response variability, and therefore less likely to lead to resistance. Novel P2Y<sub>12</sub> receptor antagonists under clinical investigation include prasugrel, AZD6140, and cangrelor.

## Conclusion

Clopidogrel and aspirin resistance are thought-stimulating and interesting research entities. Clopidogrel and aspirin resistance, although well documented in laboratory, has not been very well clinically correlated. Incorporating them into clinical practice by way of detection and treatment remains a challenge, otherwise resistance to antiplatelet therapy may remain academic curiosity.

## References

1. Fuster V, Stein B, Ambrose JA, et al. Atherosclerotic plaque rupture and thrombosis: Evolving concepts. *Circulation* 1990;82(Suppl 3):II47-II59.
2. Gurbel PA, Tantry US. Drug insight: clopidogrel nonresponsiveness. *Nat Clin Pract Cardiovasc Med* 2006;3:387-395.
3. Michelson AD, Frelinger AL, Furman MI. Resistance to antiplatelet drugs. *Eur Heart J* 2006;8(Suppl G):G53-G58.
4. Cattaneo M. Aspirin and clopidogrel: efficacy, safety, and the issue of drug resistance. *Arterioscler Thromb Vasc Biol* 2004;24:1980-1987.
5. Patrono C, Rocca B. Drug insight: aspirin resistance—fact or fashion? *Nat Clin Pract Cardiovasc Med* 2007;4:42-50.
6. Wang TH, Bhatt DL, Topol EJ. Aspirin and clopidogrel resistance: an emerging clinical entity. *Eur Heart J* 2006;27:647-654.
7. Storey RF, Newby LJ, Heptinstall S. Effects of P2Y(1) and P2Y(12) receptor antagonists on platelet aggregation induced by different agonists in human whole blood. *Platelets* 2001;12:443-447.
8. Schomig A, Neumann FJ, Kastrati A, et al. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. *N Engl J Med* 1996;334:1084-1089.
9. Leon MB, Baim DS, Popma JJ, et al. Stent Anticoagulation Restenosis Study Investigators. A clinical trial comparing three antithrombotic drug regimens after coronary-artery stenting. *N Engl J Med* 1998;339:1665-1671.
10. Bertrand ME, Legrand V, Boland J, et al. Randomized multicenter comparison of conventional anticoagulation versus antiplatelet therapy in unplanned and elective coronary stenting: The full anticoagulation versus aspirin and ticlopidine (FANTASTIC) study. *Circulation* 1998;98:1597-1603.
11. Urban P, Macaya C, Rupprecht HJ, et al. Randomized evaluation of anticoagulation versus antiplatelet therapy after coronary stent implantation in high-risk patients: the Multicenter Aspirin and Ticlopidine Trial after Intracoronary Stenting (MATTIS). *Circulation* 1998;98:2126-2132.
12. Bertrand ME, Rupprecht HJ, Urban P, Gershlick AH, CLASSICS Investigators. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting: the clopidogrel aspirin stent international cooperative study (CLASSICS). *Circulation* 2000;102:624-629.
13. Smith Jr. SC, Feldman TE, Hirshfeld Jr. JW, et al. ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/SCAI Writing Committee to Update the 2001 Guidelines for Percutaneous Coronary Intervention). *J Am Coll Cardiol* 2006;47:e1-e121.
14. Mehta SR, Yusuf S, Peters RJ, et al. Clopidogrel in Unstable angina to prevent Recurrent Events trial (CURE) Investigators. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet* 2001;358:527-533.
15. Steinhubl SR, Berger PB, Mann 3rd JT, et al. CREDO Investigators. Clopidogrel for the Reduction of Events During Observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial (CREDO). *JAMA* 2002;288:2411-2420.
16. Yusuf S, Zhao F, Mehta SR, et al. Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001;345:494-502.
17. Sabatine MS, Cannon CP, Gibson CM, et al. CLARITY-TIMI 28 Investigators. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med* 2005;352:1179-1189.
18. Chen ZM, Jiang LX, Chen YP, et al. COMMIT (Clopidogrel and Metoprolol in Myocardial Infarction Trial) Collaborative Group. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet* 2005;366:1607-1621.
19. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent

- atherothrombotic events in patients with acute myocardial infarction *Circulation* 2004;109:3171-3175.
20. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING study *J Am Coll Cardiol* 2005;46:1820-1826.
  21. Gurbel PA, Tantry US. Clopidogrel resistance? *Thromb Res* 2007;120:311-321.
  22. Grottemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non responder A pilot-study including 180 post-stroke patients. *Thromb Res* 1993;71:397-403.
  23. Angiolillo DJ, Costa Marco A, et al. Variability in Individual Responsiveness to Clopidogrel *Journal of the American College of Cardiology* 2007;49:1505-16
  24. Mueller MR, Salat A, Stangl P, et al. Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty *Thromb Haemost* 1997;78:1003-1007.
  25. Gurbel PA, Bliden KP, Samara W, et al. Clopidogrel effects on platelet reactivity in patients with stent thrombosis Results of the CREST Study. *J Am Coll Cardiol* 2005;46:1827-1832.
  26. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction *Circulation* 2004;109:3171-3175.
  27. Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome *J Thromb Haemost* 2006;4:542-549.
  28. Gurbel P A, Becker R C, et al. Platelet Function Monitoring in Patients with Coronary Artery Disease *Journal of The American College of Cardiology* 2007; 50:1822-34.
  29. Wiviott SD, Antman EM. Clopidogrel resistance: a new chapter in a fast-moving story *Circulation* 2004;109:3064-3067.
  30. Gurbel PA, Bliden KP, Zaman KA, Yoho JA, Hayes KM, Tantry US. Clopidogrel loading with eptifibatide to arrest the reactivity of platelets: results of the Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets (CLEAR PLATELETS) study *Circulation* 2005;111:1153-1159.
  31. Gurbel PA, Bliden KP, Tantry US. The effect of clopidogrel with and without eptifibatide on tumor necrosis factor-alpha and C-reactive protein release after elective stenting: results of the CLEAR PLATELETS-Ib study *J Am Coll Cardiol* 2006;48:2186-2191.
  32. Montalescot G, Sideris G, Meuleman C, et al. A randomized comparison of high clopidogrel loading-doses in patients with non-ST-elevation acute coronary syndromes: the ALBION trial *J Am Coll Cardiol* 2006;48:931-938.
  33. Thebault JJ, Kieffer G, Lowe GD, Nimmo WS, Cariou R. Repeated-dose pharmacodynamics of clopidogrel in healthy subjects *Semin Thromb Hemost* 1999;25(Suppl 2):9-14.
  34. Angiolillo DJ, Shoemaker SB, Desai B, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study *Circulation* 2007;115:708-716
  35. Angiolillo DJ. ADP receptor antagonism: what's in the pipeline? *Am J Cardiovasc Drugs* 2007 In press.
  36. Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease *J Am Coll Cardiol* 2003;41:961-965.
  37. Andersen K, Hurlen M, Arnesen H, Seljeflot I. Aspirin non-responsiveness as measured by PFA-100 in patients with coronary artery disease *Thromb Res* 2002;108:37-42.
  38. Mardikar H, Deo D, Deshpande N, Mardikar M, Steinhubl S, Mukherjee D. Variability in platelet response to single daily dose of 150mg aspirin in high risk population. *European Heart Journal*. 2007, Sep; 28(Suppl 1):664.