EuroIntervention 2011;7:263-269

Looking back into the future: desirudin in acute coronary syndromes and coronary stenting

Pascal Vranckx[1], MD; Marco Valgimigli[2], MD, PhD; Patrick Serruys[3]*, MD , PhD, FESC

1. Department of Cardiac Intensive Care and Interventional Cardiology, Hartcentrum Hasselt, Hasselt, Belgium; 2. Cardiovascular Institute, University of Ferrara, Ferrara, Italy; 3. Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands

KEYWORDS

- desirudin
- iprivask
- revasc
- acute coronary syndrome
- percutaneous coronary intervention
- direct thrombin inhibitors

Abstract

Although percutaneous coronary intervention (PCI) is a highly effective modality for the management of acute coronary syndromes, it can potentiate the existing prothrombotic state around lesion areas and lead to ischaemic complications. Adjunctive pharmacologic treatment with heparin reduces the risk of ischaemic events, but the utility of heparin is limited by its unpredictable pharmacodynamic effects and its inability to modulate fibrin-bound thrombin. Additionally, a potential risk of heparin-induced thrombocytopenia is associated with heparin use. Direct thrombin inhibitors (DTIs) have emerged as potential alternatives to heparin in patients undergoing PCI. Bivalirudin is a DTI indicated for use in PCI. Results from various studies have suggested clinical benefit associated with the use of bivalirudin, driven primarily by the reduction in bleeding risks compared with the standard treatment regimens. Of concern, however, is a significant increase in acute stent thrombosis with bivalirudin monotherapy compared with heparin plus GPIIb/IIIa inhibitors following primary PCI for ST-segment elevation myocardial infarction (STEMI). Desirudin is a highly potent DTI with greater binding affinity than bivalirudin for thrombin. This report provides a comparative overview of the pharmacology and clinical utility of desirudin and bivalirudin in the setting of PCI.

* *Corresponding author: Thoraxcenter, Ba583a, Erasmus Medical Centre, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. E-mail: p.w.j.c.serruys@erasmusmc.nl*

PCR online

DOI: 10.4244/EIJV7I2A42

Introduction

The majority of acute coronary syndromes (ACS) are caused by intracoronary thrombus superimposed on disrupted atherosclerotic plaque[1]. Platelets adhere to subendothelial proteins exposed at sites of plaque disruption where they become activated, release vasoactive and procoagulant substances, and aggregate[2]. The magnitude of the thrombotic process triggered upon plaque disruption is modulated by different elements that determine plaque and blood thrombogenicity. Exposure of tissue factor in the atherosclerotic plaque to flowing blood leads to increased thrombin generation, resulting in platelet- and fibrin-rich thrombus formation[2]. Such platelet aggregates can occur in response to spontaneous disruption of a vulnerable plaque, but they can also develop during percutaneous coronary intervention (PCI) in response to high-pressure balloon inflations and deployment of coronary stents[3,4].

Modulation of thrombotic and coagulation potential is a key factor in improving early (<30 days) clinical outcomes and in preventing complications in patients undergoing PCI[4,5]. There is clear evidence that anticoagulation in addition to platelet inhibition is effective and the combination of the two therapies is more effective than either treatment alone[6-8]. To minimise the risk of ischaemic complications during and shortly after PCI, many adjunctive antithrombotic regimens targeting thrombin generation and/or activity have been investigated and are currently in use[7-9].

Unfractionated heparin (UFH) has been widely used as the standard anticoagulant during PCI for more than two decades[5]. Heparin exerts its anticoagulant effect indirectly by binding to antithrombin, thereby dramatically enhancing the ability of antithrombin to inhibit coagulation system enzymes, particularly thrombin and factor Xa[10]. Yet there are several important disadvantages associated with the use of UFH. Due to its unpredictable, nonlinear pharmacokinetics, UFH exhibits a variable anticoagulant effect, variable binding to blood proteins and the vessel wall, and sensitivity to the inhibitory effects of platelet factor-4[10,11]. Further, the heparin-antithrombin complex is not very effective in neutralising clot-bound thrombin and, in some patients, heparin causes an immunologic thrombocytopenia (i.e., heparin-induced thrombocytopenia [HIT]), which can result in immune-mediated thrombosis[10,11]. These limitations of heparin have spurred the development of anticoagulants with different mechanisms of action, with the goal of improving outcomes and safety for patients undergoing PCI.

One approach to overcoming some of the limitations of UFH took its cue from the European leech, Hirudo medicinalis, which produces hirudin, a direct thrombin inhibitor (DTI). Direct thrombin inhibitors comprise a class of anticoagulants that bind directly to thrombin and block its interaction with its substrates[12,13]. While UFH has the potential to induce platelet aggregation, DTIs indirectly inhibit platelet activity[10,14-17]. Potential advantages associated with the use of DTIs compared with heparin include increased efficacy via the ability to bind to and inhibit fibrin-bound thrombin[12,13,18].

Several DTIs are currently approved for use by the European Medicines Agency (EMEA), namely the bivalent DTIs desirudin, lepirudin, and bivalirudin, along with the univalent DTIs argatroban and dabigatran **(Table 1)**[19-30]. Results from a recent meta-analysis of data from nearly 36,000 patients in 12 clinical trials indicated that DTIs were more effective than heparin in reducing death or myocardial infarction (MI) in patients with ACS, particularly in patients undergoing early PCI[31].

Bivalirudin (Angiox®, The Medicines Company, Parsippany, NJ, USA) is currently the most widely investigated DTI in patients undergoing PCI[32-36]. When used in place of heparin plus planned glycoprotein IIb/IIIa (GPIIb/IIIa) inhibitors, it has consistently demonstrated a reduction in protocol-defined major and minor bleeding[36-38]. Major periprocedural bleeding has been identified as an important predictor of increased mortality[36,38,39]. Whether this apparent relationship between bleeding and risk of death is a cause-and-effect relationship or merely an association based on shared risk factors remains unclear. In the Harmonising Outcomes With Revascularization and Stents in Acute Myocardial Infarction (HORIZONS-AMI) trial, bivalirudin with provisional GPIIb/IIIa inhibitors significantly reduced all-cause and cardiac mortality at 30 days and at 12 months compared with heparin and planned

Table 1. Main properties and pharmacokinetic characteristics of currently approved direct thrombin inhibitors[19-30].

	Desirudin	Bivalirudin	Lepirudin	Argatroban	Dabigatran etexilate
Route of administration and dosing	– Fixed BID SC dosing – Single IV bolus (ongoing head-to-head trial vs. bivalirudin)	IV bolus followed by continuous IV infusion	IV bolus followed by continuous IV infusion	IV bolus followed by continuous IV infusion	Orally administered capsules taken once daily
Plasma half-life	≈60 minutes	≈25 minutes	≈80 minutes	≈45 minutes	≈12-17 hours
Thrombin binding	Bivalent	Bivalent	Bivalent	Univalent	Univalent
Ki	10^{-13} M	10^{-9} M	10^{-14} M	10^{-8} M	10^{-9} M
Primary route of metabolism and clearance	Renal (80%) and renal (20%)	Enzymatic	Renal	Hepatic	Renal

aPTT: activated partial thromboplastin time; BID: twice daily; IV: intravenous; SC: subcutaneous

GPIIb/IIIa inhibitors; however, these reductions occurred at the cost of a significant increase in stent thrombosis immediately following the acute intervention (first 24 hours). There are residual concerns about increased stent thrombosis in patients with ST segment elevation myocardial infarction (STEMI) undergoing primary angioplasty and cost issues associated with bivalirudin. The other DTI that has already been tested in PCI, desirudin administered as an IV bolus, may provide a valuable alternative.

Desirudin (Revasc®, Canyon Pharmaceuticals, Hunt Valley, MD, USA) is a selective and potent thrombin inhibitor that is currently approved for prophylaxis of deep venous thrombosis in patients undergoing orthopaedic surgery[40]. Results from completed studies showed that intravenous (IV) desirudin, with or without subcutaneous desirudin, reduced ischaemic events compared with heparin in patients undergoing PCI[41-43]. More studies are under way to further investigate the clinical utility of desirudin in this patient population. In this technical report, we examine the pharmacologic basis for considering desirudin over bivalirudin in patients undergoing PCI. The framework for further clinical evaluation of this issue is presented.

Rationale for desirudin in the management of patients undergoing PCI

Desirudin, a recombinant 65 amino-acid protein, has a binding affinity for thrombin greater than 10,000 times that of bivalirudin (desirudin, ki=10-13 moles; bivalirudin ki=10-9 moles)[26,27]. The mean terminal half-life of desirudin when given as an IV bolus is approximately 60 minutes[22,44-46], allowing bolus-only administration in PCI, without the need for a continuous infusion. Desirudin has low allergic/immunogenic potential, even with repeated exposure[47,48].

A high binding affinity for thrombin, rapid onset of action, and a modestly longer half-life compared with bivalirudin are characteristics that make desirudin well suited for use in patients undergoing PCI. These pharmacologic properties may be especially advantageous in high-risk patient populations with a high thrombin load.

Furthermore, the current cost of desirudin is approximately one third of the cost of bivalirudin per procedure[49]. As the use of desirudin is expected to involve simpler administration regimens with no need for monitoring, the pharmacoeconomic considerations may be even more favourable for the use of desirudin compared with bivalirudin, provided clinical outcomes are comparable.

Overview of desirudin PCI trials

Several key interventional trials have investigated the use of desirudin in patients undergoing PCI **(Table 3)**. These studies were conducted during the early to mid-1990s when postprocedural anticoagulation was common and adjunctive use of GP IIb/IIIa inhibitors or thienopyridines was not[41-43]. Van den Bos and colleagues[41] conducted a phase II trial in 113 patients with stable angina randomised to desirudin 20 mg bolus followed by continuous infusion at a rate of 0.16 mg/kg/hr, or to UFH 10,000 U administered as a bolus and continued at a rate of 12 U/kg/hr for 24 hours. The incidence of MI and/or emergency coronary bypass surgery was higher in the UFH group compared with the desirudin group (10.3% vs. 1.4%, respectively; RR, 7.6; 95% CI, 0.9-65.6). At 24 hours post procedure, complete perfusion was present in all patients in the desirudin group compared with 91% in the UFH group, and ST-segment displacement was present in 4% of the patients treated with desirudin compared with 11% of UFH-treated patients[41].

The Hirudin with Heparin in the Prevention of Restenosis after Coronary Angioplasty (HELVETICA) trial was a pivotal, multicentre, randomised, double-blind trial that randomised 1,154 patients with unstable angina undergoing PCI to either: (1) desirudin 40 mg IV bolus plus 0.2 mg/kg/h infusion for 24 hours; (2) desirudin 40 mg IV bolus plus 0.2 mg/kg/h infusion for 24 hours, followed by desirudin 40 mg subcutaneously twice daily for three days; or (3) UFH 10,000 U IV bolus plus 15 U/kg/h for 24 hours (Table 3)[42]. The primary efficacy outcome was event-free survival (absence of death, nonfatal MI, coronary artery bypass graft [CABG] surgery, use of bailout procedures such as stenting, or second angioplasty at previously dilated sites) at 30 weeks after angioplasty. Other clinical end points included incidence of early cardiac events and measures of safety, tolerability, and luminal renarrowing. The study investigators reported that desirudin was as effective as UFH in event-free survival at 30 weeks. Importantly, desirudin was associated with a significantly reduced incidence of early clinical events (within 96 hours of PCI) compared with UFH (RR in combined hirudin groups, 0.61; 95% CI, 0.41-0.90; p=.023) **(Table 2)**. This benefit was particularly pronounced in the most unstable patients (those with Braunwald class III angina; RR in combined hirudin groups, 0.41; 95% CI, 0.21-0.78; p=.006). There were no significant differences observed between the treatment groups in the incidence of major or minor bleeding events.

Table 2. Incidence of clinical events for the intent-to-treat patient population (HELVETICA Trial). Reprinted with permission from Serruys 1995.[42]

	Desirudin-1* (n=381) n (%)	Desirudin-2¶ (n=378) n (%)	Heparin‡ (n=382) n (%)
Events at 96 hours			
Death	0	0	2 (0.5)
MI	13 (3.4)	9 (2.4)	16 (4.2)
CABG	6 (1.6)	3 (0.8)	9 (2.4)
Re-PTCA	12 (3.1)	8 (2.1)	18 (4.7)
Any event	30 (7.9)	21 (5.6)	42 (11.0)

CABG: coronary artery bypass graft; MI: myocardial infarction; PTCA: percutaneous transluminal coronary angioplasty; *Desirudin-1: 40 mg intravenous bolus +0.2 mg/kg/h intravenous infusion for 24 hours; ¶Desirudin-2: 40 mg intravenous bolus +0.2 mg/kg/h intravenous infusion for 24 hours followed by the subcutaneous administration of desirudin 40 mg bid for 3 consecutive days; ‡Heparin: 10,000 IU intravenous bolus +15 IU/kg/h for 24 hours.

Table 3. Summary of desirudin trials in patients undergoing percutaneous coronary intervention[41-43,50].

Study	Design and population	Treatments		Efficacy endpoints	Safety endpoints
		Desirudin	Heparin		
van den Bos 1993[41]	Double-blind, randomised trial in patients with unstable angina undergoing PCI (N=113)	– IV bolus: 20 mg – Continuous IV infusion: 0.16 mg/kg/h	– IV bolus: 10,000 IU – Continuous IV infusion: 12 IU/kg/h	Myocardial infarction and/or emergency coronary bypass surgery within 24 h following PTCA – Desirudin: 1.4% – Heparin: 10.3%	Major bleeding[d] – Desirudin: 5% – Heparin: 0%
HELVETICA[42]	Double-blind, randomised trial in patients with unstable angina and coronary stenosis warranting PCI (N=1154)	Group A: desirudin 40 mg IV bolus + desirudin 0.2 mg/kg/h IV infusion for 24 h + desirudin 40 mg SC BID for 3 d Group B: desirudin 40 mg IV bolus + desirudin 0.2 mg/kg/h IV infusion for 24 h + placebo	Group C: heparin 10,000 U + heparin 15 IU/kg/h 24-h IV infusion + placebo	Event-free survival at 30 wk post-angioplasty[a] – Desirudin: Group A, 68%; Group B, 63.5% – Heparin: Group C, 67.3% – No significant differences among the 3 groups (p=.61) Any event within 96 h of treatment initiation[b] – Desirudin: Group A, 5.6%; Group B, 7.9% – Heparin: Group C, 11.0% – Significant reduction with desirudin (groups A and B) vs. heparin (p=.023)	Major bleeding[c] – Desirudin: Group A, 7.7% Group B, 5.5% – Heparin: Group C, 6.2% – No significant differences between the 3 groups Minor bleeding – Desirudin: Group A, 15.1% Group B, 13.1% – Heparin: Group C, 11.3% – No significant differences between the 3 groups
Roe 2001[43]	*Post hoc* subgroup comparison analysis of PCI patients in GUSTO IIb, a prospective, randomised, multicentre, double-blind study investigating ACS patients with or without fibrinolytic therapy (N=1410)[43,50]	– Bolus: 0.1 mg/kg (≤15 mg total) – Continuous IV infusion: 0.1 mg/kg/h (≤15 mg/h total) for 3-5 d[50]	– Bolus: 5,000 U – Continuous IV infusion: 1,000 U/h for 3-5 d[50]	Death or nonfatal MI or reinfarction within 30 d – Desirudin: 6.8% – Heparin: 9.6% – No significant differences between the groups (p=.06) Death or nonfatal MI or reinfarction within 6 mo – Desirudin: 9.5% – Heparin: 12.3% – No significant differences between the groups (p=.08) Death or nonfatal MI or reinfarction within 48 h – Desirudin: 2.2% – Heparin: 3.9% – No significant differences between the groups (p=.06)	In-hospital bleeding – Desirudin: 9.7% – Heparin: 7.5% – No significant differences between the groups (p=.14)

ACS: acute coronary syndromes; IV: intravenous; PCI: percutaneous coronary intervention; PTCA: percutaneous transluminal coronary angioplasty; a. Absence of death and MI, and no further coronary interventions required; b. Incidence of death, MI, or further coronary interventions ; c. Overt bleeding, resulting in decreased haemoglobin level ≥2 g/dL, requiring transfusion of ≥2 units whole blood or packed cells; or intracranial or retroperitoneal bleeding or bleeding occurring in a major joint; d. Any intracranial and retroperitoneal bleeding, as well as bleeding at any other site requiring transfusion of ≥2 units of blood.

Finally, the GUSTO-IIb study randomised 12,142 patients with ACS to 72 hours of therapy with either IV heparin or desirudin[50]. Roe and colleagues conducted a subanalysis of 1,410 patients in GUSTO-IIb who underwent PCI while on study drug, comparing the incidence of MI and death between patients receiving desirudin versus UFH[43]. They reported that desirudin was associated with a trend toward a reduction in the primary endpoint (composite endpoint of death or nonfatal MI) compared with UFH within 48 hours post-PCI (2.2% vs. 3.9%; odds ratio [OR], 0.55; 95% CI, 0.29-1.03; p=.06). Treatment with desirudin was also associated with a lower risk than UFH of nonfatal MI at 30 days (4.9% vs. 7.6%, respectively; OR, 0.63; 95% CI, 0.40-0.98; p=.04) and at six months (6.7% vs. 9.7%; OR, 0.67; 95% CI, 0.45-0.99; p=.04), with no significant increase in procedure-related bleeding (2.8% for desirudin

vs. 2.3% for UFH; p=.53). The results from these studies in patients undergoing PCI indicate that IV infusion of desirudin provides a potentially valuable alternative to UFH in PCI.

Future development of desirudin in contemporary PCI

An urgent or early invasive mechanical (reperfusion) strategy is recommended in patients with ST segment elevation acute coronary syndromes (STE-ACS) and with non-STE-ACS considered to be at high risk for developing major myocardial necrosis, or in those at risk for rapid progression to vessel occlusion. In patients with a high thrombus burden, facilitation of coronary intervention with potent "upstream" pharmacotherapy and thrombus debulking devices appears promising. Desirudin may be well suited to this interventional strategy because of its high affinity for thrombin, (including clot-bound thrombin) and a balanced risk of major bleeding, along with the practical advantages of a single-bolus only administration and an economical price. Further research is focused on the development of desirudin for contemporary PCI. Positive results from these trials may result in establishing desirudin as the anticoagulant of choice in PCI.

First, a dose-ranging study will evaluate two dosing levels of single-bolus desirudin (30 mg or 45 mg) compared with standard regimens of UFH or bivalirudin. The primary efficacy outcome measure will include the 7-day combined incidence of death from any cause, non-fatal MI, and urgent target vessel revascularisation (coronary bypass surgery or PCI) due to myocardial ischaemia. In addition, non-CABG major bleeding occurring up to 48 hours after PCI will be assessed. Repeated blood sampling should allow for a detailed study of the coagulation profile and drug pharmacodynamics. In addition, secondary outcome measures will include area under the curve for high-sensitivity troponin. It is anticipated that this sensitive measure of myocardial damage may unmask important differences between the desirudin bolus regimens and bivalirudin. Our hypothesis is that the optimal desirudin bolus dose will provide superior ischaemic protection compared with bivalirudin owing to its higher binding thrombin-binding affinity and modestly prolonged half-life[19,22,23,26,27,29]. This study will aim to determine the optimal bolus dose of desirudin, balanced against UFH or bivalirudin, to pursue in a phase III outcomes trial.

Conclusions

Establishment of optimal antithrombotic and anticoagulant protocols remains a major goal for patients undergoing PCI, balancing the risk of ischaemic and iatrogenic bleeding complications. The short-acting nature of bivalirudin may contribute to a favourable safety profile compared with UFH plus GPIIb/IIIa inhibitors, but it may also limit its effectiveness in the prevention of acute stent thrombosis following PCI. The relative safety profiles of currently approved DTIs are unknown, as clinical studies involving direct comparisons of these agents have not yet been conducted.

Desirudin may prove to be a valuable competitor to bivalirudin in the management of patients undergoing PCI. Potential advantages of desirudin include ease of use, potential for increased efficacy owing to its increased binding affinity for thrombin, a modestly longer half-life, and its considerably lower costs. Because of these advantages, desirudin has the potential to become the anticoagulant of choice for the interventional community. Future studies will help to further characterise the advantages of desirudin in patients undergoing PCI.

Conflict of interest statement

Editorial support for this article was provided by Peloton Advantage LLC. No author received an honorarium or other form of financial support related to the development of this manuscript.

References

1. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med.* 1992 23;326:242-50.

2. Massberg S, Schulz C, Gawaz M. Role of platelets in the pathophysiology of acute coronary syndrome. *Semin Vasc Med.* 2003;3:147-62.

3. Chesebro JH, Zoldhelyi P, Badimon L, Fuster V. Role of thrombin in arterial thrombosis: implications for therapy. *Thromb Haemost.* 1999;66:1-5.

4. Harding SA, Walters DL, Palacios IF, Oesterle SN. Adjunctive pharmacotherapy for coronary stenting. *Curr Opin Cardiol.* 2001;16:293-9.

5. Popma JJ, Berger P, Ohman EM, Harrington RA, Grines C, Weitz JI. Antithrombotic therapy during percutaneous coronary intervention: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest*. 2004;126:576S-99S.

6. King SB, III, Smith SC, Jr., Hirshfeld JW, Jr., Jacobs AK, Morrison DA, Williams DO, Feldman TE, Kern MJ, O'Neill WW, Schaff HV, Whitlow PL, Adams CD, Anderson JL, Buller CE, Creager MA, Ettinger SM, Halperin JL, Hunt SA, Krumholz HM, Kushner FG, Lytle BW, Nishimura R, Page RL, Riegel B, Tarkington LG, Yancy CW. 2007 Focused Update of the 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: 2007 Writing Group to Review New Evidence and Update the ACC/AHA/SCAI 2005 Guideline Update for Percutaneous Coronary Intervention, Writing on Behalf of the 2005 Writing Committee. *Circulation*. 2008;117:261-95.

7. Bassand JP, Hamm CW, Ardissino D, Boersma E, Budaj A, Fernandez-Aviles F, Fox KA, Hasdai D, Ohman EM, Wallentin L, Wijns W. Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. *Eur Heart J.* 2007;28:1598-660.

8. Van de Werf F, Bax J, Betriu A, Blomstrom-Lundqvist C, Crea F, Falk V, Filippatos G, Fox K, Huber K, Kastrati A, Rosengren A, Steg PG, Tubaro M, Verheugt F, Weidinger F, Weis M, Vahanian A, Camm J, De CR, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U,

Silber S, Tendera M, Widimsky P, Zamorano JL, Silber S, Aguirre FV, Al-Attar N, Alegria E, Andreotti F, Benzer W, Breithardt O, Danchin N, Di MC, Dudek D, Gulba D, Halvorsen S, Kaufmann P, Kornowski R, Lip GY, Rutten F. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. *Eur Heart J.* 2008;29:2909-45.

9. Bertrand ME, Collet JP, Montalescot G. Non-ST-segment elevation acute coronary syndromes: an algorithm for decision. *Eur Heart J.* 2008;29:279-80.

10. Hirsh J. Parenteral anticoagulants: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest*. 2008;133:141-59.

11. Monreal M, Costa J, Salva P. Pharmacological properties of hirudin and its derivatives. Potential clinical advantages over heparin. *Drugs Aging.* 1996;8:171-82.

12. Weitz JI, Hudoba M, Massel D, Maraganore J, Hirsh J. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. *J Clin Invest.* 1990;86:385-91.

13. Weitz JI, Leslie B, Hudoba M. Thrombin binds to soluble fibrin degradation products where it is protected from inhibition by heparin-antithrombin but susceptible to inactivation by antithrombin-independent inhibitors. *Circulation*. 1998;97:544-52.

14. Sarich TC, Wolzt M, Eriksson UG, Mattsson C, Schmidt A, Elg S, Andersson M, Wollbratt M, Fager G, Gustafsson D. Effects of ximelagatran, an oral direct thrombin inhibitor, r-hirudin and enoxaparin on thrombin generation and platelet activation in healthy male subjects. *J Am Coll Cardiol.* 2003;41:557-64.

15. Xiao Z, Theroux P. Platelet activation with unfractionated heparin at therapeutic concentrations and comparisons with a low-molecular-weight heparin and with a direct thrombin inhibitor. *Circulation*. 1998;97:251-6.

16. Heras M, Chesebro JH, Penny WJ, Bailey KR, Badimon L, Fuster V. Effects of thrombin inhibition on the development of acute platelet-thrombus deposition during angioplasty in pigs. Heparin versus recombinant hirudin, a specific thrombin inhibitor. *Circulation*. 1989;79:657-65.

17. Badimon L, Badimon JJ, Lassila R, Heras M, Chesebro JH, Fuster V. Thrombin regulation of platelet interaction with damaged vessel wall and isolated collagen type I at arterial flow conditions in a porcine model: effects of hirudins, heparin, and calcium chelation. *Blood*. 1991;78:423-34.

18. Huhle G, Hoffmann U, Hoffmann I, Liebe V, Harenberg JF, Heene DL. A new therapeutic option by subcutaneous recombinant hirudin in patients with heparin-induced thrombocytopenia type II: a pilot study. *Thromb Res*. 2000;99:325-34.

19. Angiomax [package insert]. Parsippany, NJ: The Medicines Company; 2005.

20. Refludan [package insert]. Wayne, NJ: Bayer HealthCare; 2006.

21. Argatroban [package insert]. Research Triangle Park, NC: GlaxoSmithKline; 2008.

22. Iprivask [package insert]. Hunt Valley, MD: Canyon Pharmaceuticals, Inc; 2009.

23. Braun PJ, Dennis S, Hofsteenge J, Stone SR. Use of site-directed mutagenesis to investigate the basis for the specificity of hirudin. *Biochemistry* (Mosc). 1988;27:6517-22.

24. Di Nisio M, Middeldorp S, Buller HR. Direct thrombin inhibitors. *N Engl J Med.* 2005;353:1028-40.

25. Hijikata-Okunomiya A, Okamoto S, Wanaka K. Effect of a synthetic thrombin-inhibitor MD805 on the reaction between thrombin and plasma antithrombin-III. *Thromb Res.* 1990;59:967-77.

26. Maraganore JM, Bourdon P, Jablonski J, Ramachandran KL, Fenton JW. Design and characterization of hirulogs: a novel class of bivalent peptide inhibitors of thrombin. *Biochemistry* (Mosc). 1990;29:7095-101.

27. Parry MA, Maraganore JM, Stone SR. Kinetic mechanism for the interaction of hirulog with thrombin. *Biochemistry* (Mosc). 1994;33:14807-14.

28. Romisch J, Diehl KH, Hoffmann D, Krahl-Mateblowski U, Reers M, Stuber W, Paques EP. Comparison of in vitro and in vivo properties of rHirudin (HBW 023) and a synthetic analogous peptide. *Haemostasis*. 1993;23:249-58.

29. Talbot M. Biology of recombinant hirudin (CGP 39393): a new prospect in the treatment of thrombosis. *Semin Thromb Hemost*. 1989;15:293-301.

30. Pradaxa [package insert]. Ingelheim am Rhein, Germany: Boehringer Ingelheim International GmbH; 2009.

31. Sinnaeve PR, Simes J, Yusuf S, Garg J, Mehta S, Eikelboom J, Bittl JA, Serruys P, Topol EJ, Granger CB. Direct thrombin inhibitors in acute coronary syndromes: effect in patients undergoing early percutaneous coronary intervention. *Eur Heart J.* 2005;26:2396-403.

32. Lincoff AM, Bittl JA, Harrington RA, Feit F, Kleiman NS, Jackman JD, Sarembock IJ, Cohen DJ, Spriggs D, Ebrahimi R, Keren G, Carr J, Cohen EA, Betriu A, Desmet W, Kereiakes DJ, Rutsch W, Wilcox RG, de Feyter PJ, Vahanian A, Topol EJ. Bivalirudin and provisional glycoprotein IIb/IIIa blockade compared with heparin and planned glycoprotein IIb/IIIa blockade during percutaneous coronary intervention: REPLACE-2 randomized trial. *JAMA*. 2003;289:853-63.

33. Stone GW, McLaurin BT, Cox DA, Bertrand ME, Lincoff AM, Moses JW, White HD, Pocock SJ, Ware JH, Feit F, Colombo A, Aylward PE, Cequier AR, Darius H, Desmet W, Ebrahimi R, Hamon M, Rasmussen LH, Rupprecht HJ, Hoekstra J, Mehran R, Ohman EM. Bivalirudin for patients with acute coronary syndromes. *N Engl J Med.* 2006;355:2203-16.

34. Stone GW, White HD, Ohman EM, Bertrand ME, Lincoff AM, McLaurin BT, Cox DA, Pocock SJ, Ware JH, Feit F, Colombo A, Manoukian SV, Lansky AJ, Mehran R, Moses JW. Bivalirudin in patients with acute coronary syndromes undergoing percutaneous coronary intervention: a subgroup analysis from the Acute Catheterization and Urgent Intervention Triage strategy (ACUITY) trial. *Lancet*. 2007;369:907-19.

35. Stone GW, Witzenbichler B, Guagliumi G, Peruga JZ, Brodie BR, Dudek D, Kornowski R, Hartmann F, Gersh BJ, Pocock SJ, Dangas G,

Wong SC, Kirtane AJ, Parise H, Mehran R. Bivalirudin during primary PCI in acute myocardial infarction. *N Engl J Med.* 2008;358:2218-30.

36. Mehran R, Lansky AJ, Witzenbichler B, Guagliumi G, Peruga JZ, Brodie BR, Dudek D, Kornowski R, Hartmann F, Gersh BJ, Pocock SJ, Wong SC, Nikolsky E, Gambone L, Vandertie L, Parise H, Dangas GD, Stone GW. Bivalirudin in patients undergoing primary angioplasty for acute myocardial infarction (HORIZONS-AMI): 1-year results of a randomised controlled trial. *Lancet*. 2009; 374:1149-59.

37. Antoniucci D. The balance between bleeding and ischaemic complications in percutaneous coronary intervention practice. *Eur Heart J.* 2008;10:J21-J25.

38. Mehran R, Pocock S, Nikolsky E. Impact of different bleeding types on mortality after PCI: results from a pooled analysis of the REPLACE-2, ACUITY and HORIZONS trials [abstract]. Presented at: European Society of Cardiology (ESC) Congress; August 29-September 2, 2009; Barcelona, Spain.

39. Ndrepepa G, Berger PB, Mehilli J, Seyfarth M, Neumann FJ, Schomig A, Kastrati A. Periprocedural bleeding and 1-year outcome after percutaneous coronary interventions: appropriateness of including bleeding as a component of a quadruple end point. *J Am Coll Cardiol*. 2008;51:690-7.

40. Revasc [Annex 1 summary of product characteristics]. London, United Kingdom: Canyon Pharmaceuticals, Ltd; 2005.

41. van den Bos AA, Deckers JW, Heyndrickx GR, Laarman GJ, Suryapranata H, Zijlstra F, Close P, Rijnierse JJ, Buller HR, Serruys PW. Safety and efficacy of recombinant hirudin (CGP 39 393) versus heparin in patients with stable angina undergoing coronary angioplasty. *Circulation*. 1993;88:2058-66.

42. Serruys PW, Herrman JP, Simon R, Rutsch W, Bode C, Laarman GJ, van DR, van den Bos AA, Umans VA, Fox KA. A comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty. Helvetica Investigators. *N Engl J Med.* 1995;333:757-63.

43. Roe MT, Granger CB, Puma JA, Hellkamp AS, Hochman JS, Ohman EM, White HD, Van de WF, Armstrong PW, Ellis SG, Califf RM, Topol EJ. Comparison of benefits and complications of hirudin versus heparin for patients with acute coronary syndromes undergoing early percutaneous coronary intervention. *Am J Cardiol.* 2001;88:1403-6, A6.

44. Markwardt F, Nowak G, Sturzebecher J, Griessbach U, Walsmann P, Vogel G. Pharmacokinetics and anticoagulant effect of hirudin in man. *Thromb Haemost.* 1984;52:160-3.

45. Markwardt F, Nowak G, Sturzebecher J, Vogel G. Clinico-pharmacological studies with recombinant hirudin. *Thromb Res.* 1988;52:393-400.

46. Cardot JM, Lefevre GY, Godbillon JA. Pharmacokinetics of rec-hirudin in healthy volunteers after intravenous administration. *J Pharmacokinet Biopharm.* 1994;22:147-56.

47. Greinacher A, Eichler P, Albrecht D, Strobel U, Potzsch B, Eriksson BI. Antihirudin antibodies following low-dose subcutaneous treatment with desirudin for thrombosis prophylaxis after hip-replacement surgery: incidence and clinical relevance. *Blood*. 2003;101:2617-9.

48. Close P, Bichler J, Kerry R, Ekman S, Bueller HR, Kienast J, Marbet GA, Schramm W, Verstraete M. Weak allergenicity of recombinant hirudin CGP 39393 (REVASC) in immunocompetent volunteers. The European Hirudin in Thrombosis Group (HIT Group). *Coron Artery Dis.* 1994;5:943-9.

49. Cohen DJ, Lincoff AM, Lavelle TA, Chen HL, Bakhai A, Berezin RH, Jackman D, Sarembock IJ, Topol EJ. Economic evaluation of bivalirudin with provisional glycoprotein IIB/IIIA inhibition versus heparin with routine glycoprotein IIB/IIIA inhibition for percutaneous coronary intervention: results from the REPLACE-2 trial. *J Am Coll Cardiol.* 2004;44:1792-800.

50. A comparison of recombinant hirudin with heparin for the treatment of acute coronary syndromes. The Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) IIb Investigators. *N Engl J Med.* 1996;335:775-82.