1078-178 Application of Synthetic CDK Inhibition (Flavopiridol) as a Novel Therapeutic Strategy to Effectively Limit Common Pathways of In-Stent Restenosis: Characterization of Molecular Effects and Applicability on Drug Coated Stents

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The cellular component of in-stent restenotic lesions is mainly comprised of proliferated and migrated coronary artery smooth muscle cells (CASMC). Early cell cycle inhibition represents an attractive therapeutic strategy. Cyclin-dependent kinases (CDK) trigger and coordinate transitions between different phases of the cell cycle. Flavopiridol (FLA) is a highly effective synthetic CDK inhibitor. The main purpose of the study was to determine the efficacy and molecular effects of FLA in limiting mitogen induced CASMC proliferation and migration as well as to examine the effects of FLA coated stents on the prevention of in-stent restenosis. Results: FLA displayed potent anti-proliferative effects in CASMC, the IC50 was determined at 75 nM (BrdU ELISA, cell counting). Accordingly, CASMC CDK2, 4 and PCNA protein levels were dose dependently downregulated. Cell cycle analysis by propidium iodine flow cytometry revealed a G1 arrest in Flavopiridol treated CASMC. Given the potent anti-restenotic effects of Sirolimus coated stents, upregulated levels of endogenous CDK inhibitors such as p21 and particularly p27 may be critical for effective anti-restenotic therapy. FLA lead to a dose dependent increase of p21 and p27 protein levels in CASMC. FLA prevented p27 degradation. At concentrations of 100nM, there was neither evidence of FLA induced cytotoxicity (LDH release ELISA) nor apoptosis (ssDNA ELISA). A Boyden chamber assay revealed significant reduction of CASMC migration towards a fibronectin gradient by more than 50% at 100 nM. FLA coated stents lead to significant inhibition of CASMC proliferation in an in vitro model (FLA 137+/-12 cells, control 454+/-48, p<0.01). Initial results in an ongoing study using a rat carotid stenting model indicate reduced neointima formation in animals treated with FLA coated stents. Conclusions: Flavopiridol displays potent anti-restenotic effects. CDK inhibition by novel synthetic compounds such as Flavopiridol via drug coated stents may be an effective approach to limit neointima formation following stent placement and highlights the importance of early cell cycle inhibition as an effective tool to limit restenotic processes.

1078-179 Interferon Gamma Receptor 1 88 G/A Polymorphism and Restenosis Following Coronary Artery Stenting

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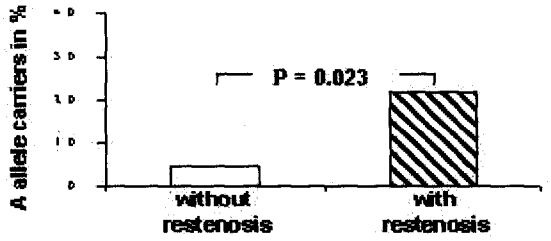
Background: Recently, upregulation of interferon gamma related genes has been found in human neointima of restenotic lesions following coronary artery stenting. The functional single nucleotide polymorphism 88G/A is located inside the signal peptide of the interferon gamma receptor 1 and results in an amino acid substitution (Val14Met). The objective of this study was to assess the influence of this polymorphism on restenosis.
Methods: This is a case control study. Subjects of both groups were nondiabetics with stenting in 2 or more vessels and follow-up angiography at 6 months. Cases were selected to have restenosis in at least 2 stented lesions and controls no restenosis at all. Each group consisted of 41 consecutive patients fulfilling these criteria. Genotyping was performed with nested PCR and restriction enzyme analysis.
Results: Cases were 67.6+12.9 years old, controls 67.5+12.0 years. None of the cases or controls were homozygous for the rarer A allele. An excess of A allele carriers was observed among cases with restenosis as compared to controls (Figure).
Conclusions: Interferon gamma receptor 1 88G/A polymorphism may have an influence on the development of restenosis following coronary artery stenting. This may be related to a shift of Th1/Th2 balance described for A allele carriage.



1078-180 Circulatory Endothelial Precursor Cells and Neural-Crest Derived Cells in Human In-Stent Restenosis

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Background: In-stent restenosis (ISR) is a major limitation of interventional cardiology. Circulatory endothelial precursor cells and neural crest derived cells might contribute to neointimal formation as reparation cells. Therefore, the objective of the present study was to evaluate CD34 and CD133 as markers of bone-marrow derived cells, and calcium-binding protein S100, glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE) and nerve growth factor receptor (NGFR) as markers for neural crest derived cells in ISR **Methods:** Atherectomy specimens from 10 patients with coronary (post-stent implantation 6±3) months), 7 patients with peripheral ISR (7±3) and 10 with primary lesions were studied by immunohistochemistry for the presence of each determinant. **Results:** Samples from ISR consistently demonstrated a homogeneous hypercellularity (1212±546 cells/mm^2 in coronary, 1061±257 in peripheral ISR, 665±98 in primary lesions). As a key finding, expression of each marker was significantly increased in ISR compared to primary lesions (each P<0.05; Table). In addition, we found positive correlations for cells of bone-marrow and neural-crest origin. **Conclusion:** The present study demonstrates the presence of intimal cells of bone marrow and neural crest origin in different types of coronary and peripheral atherosclerosis. Their significant expression in human ISR suggests an important role of these cells in this form of accelerated atherosclerosis.

	Coronary ISR	Peripheral ISR	Primary lesion
CD34	5.7±2.5	9.1±6.6	0.6±0.7
CD133	7.2±4.1	6.7±2.0	1.0±0.7
S100	10.6±6.7	8.7±4.1	1.4±1.1
GFAP	8.2±3.9	7.5±1.9	3.1±1.0
NSE	4.1±2.9	4.8±2.1	1.3±1.6
NGFR	4.5±2.3	3.7±2.7	1.1±0.7

1078-181 The Effect of Carvedilol-Loaded BiodyvYsio Stent on Neointimal Hyperplasia in a Porcine Coronary Stent Restenosis Model

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Background and Purpose: Carvedilol is a direct inhibitor of myofibroblast migration in the vascular media and adventitia and exhibits antioxidant properties. We assessed the efficacy of high concentration of carvedilol, loaded with Bio*divYsio*™ DD stent and delivered directly into the vessel wall on the inhibition of neointimal proliferation after stenting in porcine coronary arteries. **Methods:** Total loading amount of carvedilol was determined using 11mm Bio*divYsio*™ Matrix LO stents in different concentration (5mg/mL, 25mg/mL, and 40mg/mL) of carvedilol solution. Twenty-two Bio*divYsio*™ DD stents were implanted at 12 pigs, immediately after being immersed and dried in the different carvedilol or control solution. Quantitative angiographic analysis and histopathologic analysis was done 4 weeks later. **Results:** Total loading amounts of carvedilol on the Bio*divYsio*™ Matrix LO stents were 7±1 μg, 96±18 μg, and 217±34 μg from 5 mg/ml, 25 mg/ml, and 50 mg/ml carvedilol solution, respectively. Twenty-two stents were implanted and overexpanded successfully. No pig was dead during the 4-week observation. On quantitative coronary angiogram, the coronary artery diameters were not significantly different between two groups before stenting or at 4 weeks after stenting.

Table. Histopathologic analysis

	Control	5mg/mL	25mg/mL	40mg/mL	P value
Injury score	1.80±0.63	1.92±0.64	1.92±0.64	1.76±0.59	0.89
EIL area (mm^2)	7.17±0.97	7.15±0.60	6.36±0.43	6.76±1.18	0.067
IIL area (mm^2)	5.35±0.75	6.06±0.58	5.23±0.61	5.57±1.12	0.084
Lumen area (mm^2)	3.72±0.78	5.18±0.68	3.92±0.88	4.44±1.40	0.004*
Neointima (mm^2)	1.63±0.56	0.88±0.30	1.31±0.73	1.13±0.57	0.022*
Diameter stenosis (%)	30.5±10.5	14.6±5.2	25.2±13.4	21.7±14.5	0.016*

* P<0.05 between 5 mg/ml and control solution.
Conclusions: Low dose carvedilol-loaded Bio*divYsio*™ Matrix LO stents inhibit stent restenosis in porcine coronary stent restenosis model.

1078-182 Innate Immunomodulation via Transient Depletion of Monocytes by Liposomal-Alendronate Suppresses Neointimal Formation Following Balloon and Stent Injury in Rabbits

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Background: Inflammation is a major trigger for the reparative events that follow vascular injury. Excessive innate immune response correlates with neointimal hyperplasia and restenosis. We studied the impact of transient systemic inactivation of monocytes and macrophages on intimal hyperplasia following balloon injury and stent deployment.
Methods and Results: Bisphosphonates encapsulated in liposomes are phagocytosed by and specifically inactivate macrophages. Endothelial or smooth muscle cells that do not imbibe these formulations are left intact. Rabbits fed a hypercholesterolemic diet underwent bilateral iliac balloon denudation and stent deployment. Suppression of blood monocytes from 94±18 to 18±15 / ml (p<0.01) was observed 48 hours after injury and intraarterial injection of liposomal alendronate (3 mg/kg). Suppression was transient with return to normal levels a week after injury (flow cytometry for CD14+ cells). The reduction in blood monocytes was associated with reduced infiltration by tissue macrophages (at 6 days, RAM-11 immunostaining), suppressed arterial cellular proliferation (Ki-67 immunostaining) and neointimal formation at 28 days. Intimal area was reduced by 47% (3.88±0.93 to 2.08±0.58 mm2), lumen area was increased by 24% (2.87±0.44 to 3.57±0.65 mm2) and stenosis (%) was reduced by 25% (34± 4 to 25± 4%) (mean±SD, n=16, p<0.01 for all 3 parameters).
Conclusions: Innate immunity triggers vascular repair; and when extreme, as in stented

vessels, can lead to excessive proliferation. Transient systemic inactivation of monocytes and macrophages by liposomal alendronate reduced neointimal formation and restenosis in the stented hypercholesterolemic rabbit model in a manner that can be used independent of stent number, position, or overlap and with possible multiple dosing.

1078-183 **The Endothelial Nitric Oxide Synthase (Glu298Asp and -786T>C) Gene Polymorphisms Are Associated With Coronary In-Stent Restenosis**

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Background Coronary stent deployment is a major advance in percutaneous treatment of ischaemic heart disease, but 10-40% of patients still develop angiographic restenosis by 6 months due to neointimal hyperplasia. Patient-specific factors, including genetic factors, can contribute to this process. We have conducted a prospective study to examine the involvement of genetic risk factors (eNOS, ACE, MMP-3, IL-6, and PECAM-1) in restenosis following coronary stent deployment.
Methods 226 patients who underwent elective coronary artery stenting to *de novo* lesions in native coronary arteries were studied. 205 (90.7%) patients were restudied by coronary angiogram at 6 months and the stented lesions were assessed with automated quantitative angiography system. Genotype was determined by PCR and restriction enzyme digestion. **Results** Restenosis rate, defined as ≥50% diameter stenosis, was 29.3%. The overall genotype frequency distributions were in Hardy-Weinberg equilibrium for all variants. Carriers of the 298Asp allele of the eNOS Glu298Asp polymorphism showed a higher frequency of restenosis with an odds ratio of 1.88 (95%CI:1.01-3.51, p=0.043) compared to 298Glu homozygotes. Carriers of the -786C allele of the eNOS -786T>C polymorphism also showed a higher frequency of restenosis with odds ratio of 2.06 (95%CI:1.08-3.94, p=0.028). Other studied genes did not show significant association with coronary in-stent restenosis.
Conclusions In patients with coronary artery disease, the possession of the 298Asp and -786C variants of the eNOS gene is a risk factor for coronary in-stent restenosis, demonstrating the importance of the nitric oxide system in restenosis.

ORAL CONTRIBUTIONS

805 Drug-Eluting Stents

Monday, March 31, 2003, 9:15 a.m.-10:30 a.m.
McCormick Place, Room S401

9:15 a.m.

805-1 **Two-Year Follow-Up of the RAVEL Study: A Randomized Study With the Sirolimus-Eluting Bx VELOCITY Stent in the Treatment of Patients With De-Novo Native Coronary Artery Lesions**

Marie-Claude Morice, Patrick Serruys, Costantino Costantini, Egon Wuelfert, William Wijns, Jean Fajadet, Antonio Columbo, Giulio Guagliumi, Ferenc Molnar, Ernesto Ban Hayashi, Jose Eduardo M. Sousa, Marco Perin, on behalf of the RAVEL Trial Investigators, Institut Cardiovasculaire Paris Sud, Massy, France

Background: Sirolimus, a macrocyclic antibiotic and immunosuppressant, causes late G1 cell cycle arrest. A pilot study using Sirolimus-eluting stents (SES) to treat coronary artery lesions demonstrated almost no neointimal hyperplasia at 12 months. Results from this study were used to design RAVEL, a multicenter, double blind, two-arm, randomized study assessing safety and effectiveness of the Sirolimus-eluting Bx VELOCITY™ stent versus the uncoated Bx VELOCITY™ stent (18 mm). **Methods:** The primary endpoint was angiographic late loss at 6 months. The secondary endpoints were major adverse cardiac events, target vessel revascularization (TVR), target lesion revascularization (TLR) and restenosis rate. Inclusion criteria: stable or unstable angina, single treatment-de novo lesions < 18mm long, 2.5-3.5 mm diameter. Clopidogrel or Ticlodipine was continued for up to 8 weeks following a loading dose. **Results:** From August 2000-December 2000, 238 patients were enrolled at 19 sites in Europe and South America. Baseline demographics and lesion characteristics were evenly distributed. No acute, subacute or late thrombosis occurred. One year results.

	Sirolimus	Control	P-value
Reference diameter (mm)	2.60	2.64	NS
MLD Post-procedure (mm)	2.43	2.41	NS
MLD Follow-up (mm) – 6 month	2.42	1.64	.0001
Late loss (mm)	0.01	0.80	.0001
Restenosis rate (%)	0.0	26.1	.0001
TLR-free – 1 year	99.2	86.0	.0001
TVR-free – 1 year	98.3	84.3	.0001
TVF-free – 1 year	95.8	80.3	.0001
MACE-free – 1 year	94.1	81.2	.0001

Conclusion: The QCA data demonstrate virtually no neointimal in-stent proliferation with event-free survival of 94% at one year in the SES treatment group. Two-year clinical follow-up data will be presented.

9:30 a.m.

805-2 **Cost-Effectiveness of Sirolimus Drug-Eluting Stents for the Treatment of Complex Coronary Stenoses: Results From the Randomized SIRIUS Trial**

David J. Cohen, Ameet Bakhai, Chunxue Shi, Louise Githiora, Ronna H. Berezin, Ronald P. Caputo, Charles O'Shaugnessy, Martin B. Leon, Jeff Moses, Richard E. Kuntz, on behalf of the SIRIUS Investigators, Beth Israel Deaconess Medical Center, Boston, MA, Harvard Clinical Research Center, Boston, MA

Background: Previous studies have demonstrated that sirolimus drug-eluting stents (DES) dramatically reduce angiographic and clinical restenosis compared with conventional stenting. However, the cost-effectiveness (C/E) of DES in routine clinical practice is unknown. **Methods:** We prospectively measured medical resource utilization and cost for 1100 PCI patients randomized to either sirolimus DES or bare stents (BS) as part of the SIRIUS trial. Costs were assessed from the U.S. societal perspective, and each DES was assumed to cost $3000/stent. **Results:** Resource utilization and 1-year cost data for the first 400 randomized patients are displayed below (see Table). Although DES reduced the rate of target vessel revascularization by 62% and follow-up costs by ~$1500/pt, total 1-year costs remained higher with DES ($14,245 vs. $13,642, p<0.001). The incremental C/E ratio for DES compared with BS was $5542 per repeat revascularization (RepRev) avoided-- similar to the C/E of BS for de novo lesions or brachytherapy for diffuse intrastent restenosis (~$5000 - $10,000 per RepRev avoided).
Conclusions: 1) In a representative population of PCI patients, sirolimus DES improved clinical outcomes but increased overall health care costs by ~$600/pt compared with BS. 2) Nonetheless, considering the quality of life benefits of avoiding restenosis, the C/E of DES compares reasonably with other accepted interventional techniques. 3) Complete 1-year data on the full 1100 pt trial will be available by 3/03.

	Sirolimus Stent	**Bare Stent**	**P-value**
Number of stents/patient*	1.1± 0.4	1.1± 0.5	NS
Cath lab cost	$6225±1385	$4179±1018	<0.001
Initial hosp. Cost	$10,704±2139	$8611±1913	<0.001
1-year outcomes			
Repeat revasc. (target vessel)	6.8%	17.6%	<0.001
Repeat revasc (any vessel)	12.6%	23.3%	0.006
F/U cost	$3541±3204	$5040±5098	<0.001
1-year total cost	**$14,245–3869**	**$13,646–5546**	**<0.001**

* Adjusted to account for the availability of stent lengths up to 33mm in clinical practice

9:45 a.m.

805-3 **One-Year Follow-Up of the SIRIUS Study: A Randomized Study With the Sirolimus-Eluting Bx VELOCITY in the Treatment of Patients With De-Novo Native Coronary Artery Lesions**

David R. Holmes, Jr., Martin B. Leon, Jeffrey W. Moses, Jay Midwall, Mel Clark, Igor Palacios, Mark Bates, John Lopez, Alan C. Yeung, Richard E. Kuntz, on behalf of the SIRIUS Trial Investigators, Mayo Clinic, Rochester, MN

Background: The Sirolimus-eluting stent (SES) is emerging as a potential solution to the problem of restenosis in a variety of patient populations. The SIRIUS trial is a 1058 patient, multicenter, double blind study conducted in the US comparing the Sirolimus-eluting stent (SES) to the uncoated Bx VELOCITY (control). The interim results of the first 400 patients, previously presented, demonstrated the safety and effectiveness at 9 months. Longer term follow-up of these patients is planned at 12 months, and annually out to five years to confirm the durability of this treatment of de novo native coronary artery lesions. **Methods:** From February to August, 2001, 1058 patients were enrolled at 53 centers. The primary endpoint was target vessel failure (TVF) at 9 months. Other clinical, angiographic and IVUS endpoints were also evaluated. Baseline demographics on the entire cohort revealed a mean age of 62 years, 26.4% diabetics, 41.6% with multivessel disease, 30.6% had a prior MI. The average lesion length was 14.4mm and the reference vessel diameter was 2.80mm. **Results:** Nine month clinical follow-up results are presented below (Table 1). Accordingly, sirolimus-eluting stents appear to be safe and effective at 9 months. Twelve-month clinical follow-up will be presented.