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Preclinical Evaluation of Drug-Eluting Stents for Peripheral Applications

Recommendations From an Expert Consensus Group

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Drug-eluting stents implanted in the coronary arteries substantially improve long-term outcomes for restenosis. The utility of the stents in peripheral atherosclerosis is under evaluation. Drug-eluting stent evaluation with coronary arteries appears to be an excellent method for evaluating human safety end points. Predicting clinical efficacy remains unclear, however. Because drug-eluting stents are undergoing development for human peripheral arteries, safety and efficacy questions arise in much the same context as they did for the coronary arteries. As they did with the coronary arteries, the clinical, scientific, regulatory, and commercial communities are seeking acceptable criteria for peripheral device evaluation. Substantial differences in stent requirements and biological effects for bare metal peripheral stents depend on implant site. Different anatomic locations under evaluation include femoral, renal, and neurologic arterial stents, and other peripheral applications such as outflow veins of dialysis arteriovenous fistulae are being evaluated. Peripheral in-stent restenosis is less of a problem in carotid and aortoiliac stents and in iliac veins and in the cavae. In general, the benefit:risk ratio for drug-eluting stents may be less for peripheral stents. It is for this reason that peripheral stent performance should be evaluated carefully.

This document presents an integrated recommendation set for evaluating drug-eluting stents in peripheral vessels with preclinical models. The recommendations encompass study design, experimental performance, and histopathologic evaluations and emphasize the need to evaluate safety and efficacy at multiple points in time. The present document is a consensus of clinical, academic, and commercial groups—all experts in the evaluation of preclinical investigational or interventional devices. Because preclinical peripheral studies

are less well understood than are coronary models, the recommendations do not prescribe a single method for device evaluation; they instead provide broad suggestions for evaluation. Peripheral vascular knowledge will increase as correlations of preclinical with clinical data become available. Future versions of this document will necessarily reflect such enhanced knowledge.

Drug-eluting stents are implantable devices that present or release single or multiple bioactive agents into blood vessels after implantation. A formal definition for the drug-eluting stent is as follows: A stent combined with therapeutic agent(s) physically affixed, often by embedding the agent in a carrier coating such as a polymer matrix and/or by directly applying the agent to the stent surface or within the stent. Following implantation, the therapeutic agent(s) is/are released into the blood stream as well as locally into tissue adjacent to the stent.

Drug formulations, matrices, and release are as described in the 2002 coronary artery guidelines.¹

One major purpose of drug-eluting stents is to limit neointimal hyperplasia. Before clinicians consider undertaking preclinical studies, they should have strong reasons for considering stents and agents for an application such as this. Preclinical studies should follow cell culture work or work that suggests efficacy in other applications (eg, malignancies). In vitro testing should be a fundamental requisite for characterizing the elution kinetics of the agent from the stent and devices.

Peripheral stent drug dosing and kinetics should be studied as they are with coronary stents. The proposed clinical doses and stent kinetics should be justified by in vivo preclinical

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TABLE 1. In Vitro and In Vivo Drug Release Characteristics

1. In vitro half-life estimate, $t_{1/2}$, where half of all available drug has been released
2. In vitro characterization of elution, either to plateau or point at which 80% of drug substance has been released
3. In vivo peak tissue and blood concentrations, and a time-course graphic of drug remaining in stent
4. In vivo half-life estimate, $t_{1/2}$
5. Dose range showing subtherapeutic to toxic levels; safety margin should be estimated and justified by data
6. Drug concentrations in blood, peripheral artery, and tissue beneath stent over time points from immediately after implantation until near-complete drug elution; drug concentration in tissue supplied by stented artery, whether in extremities, liver, kidney, brain, or lung as measured at necropsy
7. In vivo pharmacokinetics
a. Additional time points to more fully characterize drug release into artery tissue
b. Drug levels in arterial tissue proximal and distal to stent
c. Drug levels in organ and tissue proximal and distal to stent

data. Multiple doses should be examined in peripheral stents to determine a safe range and, if possible, to determine where toxicity occurs. Ideally, a toxic dose should be 3 to 10 times higher than the anticipated clinical dose giving a comparable safety margin.

Systemic and “downstream” drug effects and toxicity that occur with higher dosages in peripheral stents should be carefully documented. Intravenous injection studies should be performed to estimate systemic toxicity. Long-term stented animal studies should be performed with monitoring plasma drug levels. Assessment should be per the previous guideline document, and at a minimum should include nephrotoxicity, hepatotoxicity, neurotoxicity, and possible immune compromise. Perfusion magnetic resonance imaging (MRI) and positron emission tomography (PET) also may be useful in evaluating the central nervous system for carotid stenting, in addition to Doppler ultrasound of extracranial vessels.

The characterization of in vitro and in vivo drug release should follow that described for drug-eluting stents for coronary application (Table 1). In vivo drug release can be characterized according to the guidelines for coronary stents. As with these guidelines, ≥ 6 stents per time point should be used. Blood levels should be used to estimate a “systemic pK” for the drug.

Additional measurements for estimating in vivo pharmacokinetics can be obtained. These include adding time points to better characterize drug release into arterial tissue, better defining a reasonable safety margin dose, and estimating drug levels in arterial and other tissues proximal and distal to the stent. These measurements also are summarized in the coronary artery guidelines. As with those guidelines, in the case of bioerodable coatings, information about disappearance should be presented.

Peripheral stents appear to have an increased risk for fracture in proportion to increase in their diameter. Fracture appears to occur in both drug-eluting and bare metal stents.² A polymer-based drug-eluting stent with fracture might theoretically enhance local fluid, corrosion, and possibly stent fracture. A systematic investigation of stent breakage should be included in research done on peripheral drug-eluting stents.

Stent drug-release characterization both in vitro and in vivo should be studied before undertaking human clinical testing and should be understood before performing animal testing. The reproducibility of results should be established to ensure

the interpretability of subsequent clinical and animal evaluations. Drug elution and its characterization are inexact and technically difficult, but careful attempts with documentation should be made regardless. The important drug-eluting stent applications are for in-stent restenosis, and experience suggests that testing should determine the healing response for single and overlapped stents.

Animal Models

Healthy animal models are generally accepted to be useful for understanding the mechanisms of the arterial response to injury. They also are useful in understanding the safety of arterial stenting. The utility for understanding efficacy in clinical trials remains uncertain. Proof of early concept occurs commonly in animal models, and this includes toxicity and response to the mechanical prosthesis. Actual efficacy and safety can be proven in human trials or surmised from animal studies when it is shown that human data are well reflected by such preclinical data. Preclinical trials should resemble clinical trials in establishing important data and drawing conclusions as best as possible.

The ideal animal model for the evaluation of peripheral drug-eluting stents is uncertain and, at present, less well understood than it is for coronary models. Drug deposition and pharmacological responses vary with arterial site and lesion morphology. Devices used for normal peripheral vessels in animals may be used as models for the design of devices for human peripheral clinical application. Histopathology suggests that the peripheral arteries of domestic crossbred swine and the iliac arteries of rabbits may be best suited because their size and access are similar to those of human vessels. Both exhibit substantial elastin and show comparability of the intima, media, and adventitia. The injury response with neointimal formation of peripheral porcine or rabbit vessels is not presently well understood.

Device selection and testing should take place in vessels with comparable dimensions, and stents should be appropriately sized to the artery in preclinical studies. Too much mechanical stent injury confounds safety and efficacy results. To obtain a comparable model in larger-vessel studies, it may be necessary to ligate vascular branches. Safety and efficacy should be examined in a comparative study with several time points. All tissue surrounding the stent, including muscle, nerves, and vessels, should be examined for toxic effects,

especially if the drug used is known to be toxic to certain types of tissues and cells.

As with the coronary arteries, the most important safety concern in peripheral drug-eluting stents is acute and sub-acute stent thrombosis. All animals that experience adverse consequences, limb ischemia, death, or other untoward clinical events should be examined and their stent status carefully documented. Because standard stent practice in patients entails oral aspirin plus clopidogrel or ticlopidine, these agents should be administered throughout the preclinical study.

The underlying tissues (eg, muscular or elastic arteries, veins) in superficial femoral artery and vascular access stents may respond differently to drug-eluting stents. One limit in model planning is that peripheral porcine arteries appear not to develop neointima as vigorously as do coronary arteries (Schwartz, Edelman, Carter, and Chronos, unpublished observations, 1990–2000). Accordingly, when possible, stent testing in the vascular bed or in the same type of artery or vein where stents will be used clinically is preferable. Similarly, the use of maximum-length stents at clinical doses/unit area, total doses, and release rates intended for clinical use is indicated. Drug toxicity, thrombus, and end-organ infarct are of particular concern when stenting renal and carotid arteries.

Porcine Peripheral Artery Model

The preferred porcine model is the normocholesterolemic domestic crossbred, or mini-swine artery. Stents may be placed in the carotid, renal, or iliofemoral systems. Stents should be appropriately sized for the artery (stent:artery ratio of between 1.0 and 1.1) and implanted into arteries with no previous injury.

Rabbit Iliac Artery and Other Models

The Expert Consensus Group considers the rabbit iliac artery to be less acceptable as a model for peripheral studies because the vessels are smaller and the reaction to injury is less well defined. The canine model has limited inflammatory responses and may be less sensitive as a safety model. Other species that may provide longer and larger vessels in the neck and legs include the goat and sheep. Calf arteries grow quickly and larger vessel diameter changes occur over short time periods (A.P. Moody, unpublished data, 1991), which may confound researchers conducting studies of ≥ 6 months' duration. Unfortunately, experience with and knowledge of stenting in these alternative species are limited or unknown at this time.

Drug-Eluting and Control Stents

Only 1 stent should be implanted per artery except when questions of stent overlap or multiple-stent dosing are considered. Stents may be placed in multiple different arteries in the same animal, and an adequately controlled study should include bare, carrier-only, and carrier-plus drug-eluting stents. The stents should be appropriately sized (stent:artery ratio of $\leq 1:1$). When possible, stents should be tested in the arterial bed corresponding to that in which the stents will be clinically deployed. This consideration includes the need in preclinical studies to implant the stents in arteries of similar

type as much as possible, whether it is muscular, elastic, or conduit.

Preclinical trials must be adequately controlled. Polymeric materials for drug elution frequently affect the arterial repair process, generally in a toxic manner. When a polymer or carrier of any sort is present, it is imperative to include as controls stents that are coated with polymer alone, especially because polymer coatings that do not contain drug may react differently from coatings that are devoid of drug after complete release. This may reflect different surface characteristics (eg, porosity or texture), especially when matrix-type devices are used. In addition, changes in the drug-coating procedure should merit additional studies of the polymer alone because the coating procedure may substantially change the biological activity of the device. When drug is bound directly to a stent, the stent without drug can be a satisfactory control, although any changes to the surface of the metal should be applied to the control devices. When drugs are attached directly to metal stents without a polymer, the stent metal is activated by creating a metal oxide layer. In this case, the control devices for testing should be subject to the identical steps used in the manufacture of drug-eluting stents before attachment of the active drug. This should include identical cleaning, packaging, and sterilization procedures.

Testing Boundaries

Stent Size

If numerous stent sizes are to be manufactured and studied in human clinical trials, then the longest and largest-diameter stent and the longest and smallest-diameter stents should be tested. With even larger stent varieties ("families"), testing intermediate sizes or configurations is warranted. For long peripheral drug-eluting stents, the downstream effects on vessel wall healing and histology and morphology may be exaggerated as compared with "upstream" vessel walls, given the additive drug concentrations downstream. Peripheral vascular drug-eluting stents are potentially longer and have larger diameters than do coronary stents and may carry higher total drug dosages than do coronary stents, making it desirable to investigate drug effects and healing along the stent length, especially with long stents, and in tissues downstream from the stents. Histopathologic observations and histomorphometric analyses should be summarized and compared among proximal, mid-, and distal stent locations in long stents. It may be necessary to cut 5 instead of the usual 3 sections used in coronary artery applications.

Overlapping Stents

Stent overlap is sometimes needed during clinical implantation. Because overlapping drug-eluting stents may cause an additive or combined effect from the 2 stents, intentional overlap should be evaluated in preclinical studies. The overlap distance should be roughly one third the length of a stent, or ≤ 4 mm, and the number of overlapping stent-implant pairs evaluated should be no less than 5. Histopathologic processing should be done with sections taken from the reference segments, the single (nonoverlapping) stented region, and the overlapping stented region.

Sampling Time Points and Size

Stent efficacy or relative vascular patency at the stent site should be evaluated. Neointima should not be obstructive. Thrombus must be absent and data should be obtained early (3 or 7 days) to determine subacute thrombosis risk and early histopathology. Long-term neointimal thickening and other arterial effects should be monitored at 28 days and at least 1 late time point. The late monitoring point (3 or 6 months) depends on when healing and drug release both are complete for the model chosen (for rabbits, see references 3 through 5; data for other species are pending). Note that the US Food and Drug Administration typically recommends monitoring for ≤ 6 months. Three-month follow-up data are in general acceptable for initiating clinical feasibility trials if no adverse findings are noted at the time. Six-month data should be available at the time of Investigational Device Exemption submission for a pivotal study. These later time points are especially important given the impact of peri-stent late remodeling as an additional cause of the peri-stent effects that would affect the clinical outcome.

Long-term time points for animal studies (≥ 6 months) are important, but results may be less rigorously interpreted until global understanding is obtained about the relationship between animals and humans at longer implantation times. Such studies on coronary and peripheral vessels by several investigative groups are pending or under way. The choice of a long-term end point becomes more complicated when dealing with drug-eluting stents. Whereas injury after balloon angioplasty or stent implantation may peak and then resolve over weeks to months, the presence of a drug might change that dynamic.

Arterial reaction to the device and general animal health should be reviewed for a practical multiple of the drug residence time within the primary target tissue. Elution observation thus corresponds to 4 elution half-lives in the tissue, after the drug is no longer measurable from the release devices. Because the tissue will be nearly devoid of drug within 4 half-lives, waiting for this period of time is thought to be appropriate. Another important time point is drug metabolic half-life, a parameter that is less easily measured.

The number of stents for study should be determined by a power calculation for the predetermined expected difference in key parameters. Sample size power calculations are not yet well defined; therefore, estimation is appropriate. Typically, 7 to 10 drug-eluting stents per time point are satisfactory in most peripheral models, but this amount depends on the variability seen in the data; however, this number should be sufficient to draw conclusions. One method for analyzing coronary artery results uses a comparison of regression models. This model should be applicable to peripheral vessel studies.⁶

Implantation Procedure

Veterinary anesthesia should be established per accepted standard, in compliance with the local Institutional Animal Care and Use Committee and the Association for Assessment and the Accreditation of Laboratory Animal Care. The choice of surgical technique for the implantation procedure including cutdown and catheters, wires, and other procedural

equipment may be at the investigator's discretion when it is in compliance with accepted standards.

Stent-Related Antiplatelet Medication

All animals may receive antiplatelet therapy (aspirin plus clopidogrel or ticlopidine) daily, beginning 1 day before the procedure and continuing for the duration of survival. Antiplatelet drug use and doses should reflect the drug regimen planned for a clinical trial unless the therapy is not effective in the test animal species. This should be determined when species are chosen for study in which the antiplatelet agent efficacy is not known.

Autopsy/Necropsy Evaluation

Necropsy for both scheduled and unscheduled death is important in the evaluation of stents. All deaths should be carefully documented and explained, especially in the peripheral vessels of pigs and rabbits because death occurring later than the first 24 hours is rare. All unexpected deaths should be closely examined by necropsy, gross evaluation, and histopathologic examination with special attention given to the stents as possible causes of death. Thrombus within the stent should be subjected to histopathologic examination and it should be determined whether the thrombus occurred either pre- or postmortem. Variegated platelet-fibrin component, clot layering, clot adhesion to the vessel wall, and presence of polymorphonuclear leukocytes with cellular organization and maturation indicate premortem stent thrombosis.

Necropsy should be performed by qualified personnel to determine the cause of death for all animals dying after entry into the study, regardless of whether or not the study animal completed the allotted survival time. An expert opinion should be rendered as to the cause of death when it does not result from euthanasia. The performance of all stents in such early or unexpected deaths should be determined, recorded, and reported. The target organ or tissues of the vessel bed downstream of the stent implants should be examined for any evidence of infarct (and distribution), as well as for any fibrosis, especially in the perivascular region.

Histopathology should be performed on all implanted stent-artery segments, including segments in those animals dying any time after the implantation procedure has begun. All implanted stents should be sectioned, regardless of how long they were implanted. The thoracic cavity (pigs) or abdominal cavity/retroperitoneum (rabbits) should be examined for effusion, inflammation, infection, perforation, or other systemic problems.

Tissue Processing and Fixation

Fixation is important for preserving artery size and shape. The precise method should be determined by the fixative required and analysis to be completed. Pressure perfusion fixation should be performed at ≈ 100 mm Hg. Immersion fixation should be performed at a volume that is sufficiently large to allow complete and rapid fixative percolation through the tissue and without alteration of the tissue shape. After removal, the target organs, tissues, or limbs containing the stent should be sectioned transaxially (short axis sections) at minimum intervals of 1 cm. These sections should be exam-

ined grossly for target organ or muscle infarction. All such infarctions should be included in the final report.

Histopathologic Stains, Histopathology, and Histomorphometry

Histopathology and histomorphometry are key to determining stent performance and effects, both positive and negative. Plastic or epoxy embedding is strongly recommended, as paraffin sectioning with strut removal disturbs tissue and cell relationships.⁷⁻⁹ The use of hematoxylin and eosin, elastin, and trichrome (preferably Masson) stains is recommended. More specific cellular responses require specialty stains and immunohistochemical techniques. Several sections should be taken to examine the entire stent, including the proximal and distal segments, and the adjacent/affected tissues. A pathologist or other individual with extensive and specialized experience in microscopic examination of stented arteries must be the primary reviewer of tissue and stents, and should either perform or closely supervise other individuals performing measurements on the arterial sections. Such observations should be blinded to treatment group and should include proximal, middle, distal, and proximal and distal reference artery (minimum distance, 10 mm).

Clinical and Blood Parameter Evaluations

Animal well-being also is observed after stent implantation. Clinical features include normal physical signs and blood parameter measurements. The drug-eluting stent should have minimal effect on physical signs and clinical parameters. The following should be documented in all animals: general health (daily record), body temperature at follow-up, and body weight over the course of the study. In the event of unscheduled death of the study animal, a postmortem evaluation of any relationship to the stent should be sought, as in porcine coronary implants. In the case of peripheral artery stents supplying the extremities, careful clinical observations of gait, stance, atrophy/tissue changes, and ambulation should be considered as possible screening methods for detecting stent complications or developing leg problems. Other clinical signs may be important for functional assessment or warrant consideration when stenting critical vessel beds such as the carotid (neurological status) and renal (kidney output) arteries.

Blood parameters should be tested for findings that are suggestive of allergy or hepatic or renal dysfunction. These measures should be taken at baseline and at euthanasia and include a complete blood count with differential blood count, liver enzymes (alanine aminotransferase, aspartate aminotransferase), and creatinine. Specific chemical tests may need to be performed or monitoring laboratory parameters assayed if idiosyncratic effects on tissues and cells are suspected or if clinical symptoms develop.

Arteriography and Intravascular Ultrasound

Peripheral vascular arteriography can yield important information about the arterial lumen and patency within the stent and should be performed immediately before euthanasia, with special attention paid to peri-stent effects. Intravascular ultrasound (IVUS) may be performed in a minority of stents

to look for peri-stent effects and neointimal formation. Peri-stent effects are those including the 5 mm beyond the stent ends. IVUS is optional in the stented and reference segments and may be performed in a minority of cases or in more cases if desired by the investigator, as long as procedure-induced injury to the stented segment is avoided. IVUS can help answer questions concerning peri-stent effects when visualized by arteriography or subsequent pathological evaluation. Routine IVUS in all animals may damage the endothelium and stented artery, however, and should be considered only after careful consideration.

Stent Evaluation

Simple visual description of the histopathology is inadequate as the sole evaluation. A rigorous semiquantitative approach with defined scales for device performance and safety evaluation is more informative.

Semiquantitative Histopathology

Inflammation and Injury

Inflammation by histopathologic evaluation might include an injury score (value 0 to 3) at each stent strut site, an inflammation description (absent, or cell types and location), and an inflammation score (value 0 to 3) for the overall vessel as well as the adventitia media, neointima, and at stent strut sites (see, eg, reference 10). When possible, cell density in specific tissue compartments should be recorded as number of cells per area.

Angiogenesis and Other Histopathology

Angiogenesis can be assessed (scored 0 to 3) and reported for the adventitia, media, and neointima.¹¹ Other histopathologic features should be sought in the adventitia, media, and neointima and assigned a value of 0 through 3 or be described quantitatively as the number and size of vessels per unit area. These observations include fibrin or fibrinoid deposits, hemorrhage, and necrosis.

Observational Histopathologic Data

Endothelialization, Reendothelialization and Vessel Healing

A stented vessel should be considered healed when it shows endothelialization or a healthy-appearing layer of near-complete periluminal cells. Endothelialization should be recorded as absent, partial, or complete in all sections. A semiquantitative analysis can be performed and presented as the percentage of circumference covered by endothelium. The time to reendothelialization should be estimated. Scanning electron microscopy from ≥ 3 stents is recommended to assess endothelial recovery. The Expert Consensus Group suggests that a completely healed vessel exhibits reendothelialization and no evidence of fibrin, fibrinoid deposits, excessive inflammation, or hemorrhage.

Stent Strut Position, Apposition/Malapposition, and Adjacent Tissue

Other observations should include the extent to which stent struts are apposed to the vessel wall (percentage of wires in contact) and covered by tissue or endothelium (as a percentage). A subjective description also should be created for adjacent tissue, including medial necrosis or thinning, loss of

cellularity, and hyalinization. Stents should be observed carefully to determine whether strut malapposition exists.

Organ Evaluation and Histopathology

Whether renal, cerebral, visceral, or muscle, tissue histology directly beneath, distal to, and supplied by the stented artery should be observed and recorded as normal or abnormal and same or different from control stents. Histopathology is a standard. Because of its sampling nature, however, it may not be as sensitive as is necessary to detect stent-induced organ damage, particularly in the extremities and in solid organs. Vasoconstriction or embolic phenomena induced by the stent may be a concern with certain drug-eluting technologies, and it is therefore recommended that an evaluation method capable of 3D analysis be used in a fraction of devices. This method might take the form of computed tomography or MRI scanning of the intact organ before histopathology, and if abnormalities are found, the histopathology may be guided by such imaging methods. Serial complete gross sectioning at 5-mm increments with a gross dissection blade also can be used to locate organ pathology. Specific additional but optional end-organ evaluations follow.

Extremities

The postmortem evaluation of limb muscles may be performed with radioactive microspheres. Perfusion assessment would include measured radioactive counts of homogenized tissue or MRI methods.^{12,13} In vivo muscle studies could be performed with standard radioisotope-based perfusion measures. Histopathology of distal muscle should be evaluated for abnormal findings of fiber size variation, nuclear abnormalities, fiber angulation, pyknosis of nuclei, and fiber atrophy.

Kidneys

Radionuclide renal studies in vivo may be performed with labeled microspheres or radioisotopes such as ^{99m}Tc dimer-captosuccinic acid, used for comparative imaging of stented versus nonstented kidneys to assess parenchymal abnormalities. Renal histopathology should be examined for evidence of glomerulonephritis, sclerosis, hemorrhage, fibrosis, and inflammation. Attention should be paid to glomerular hyper- and hypocellularity and mesangial appearance, as well as to the search for interstitial fibrosis.

Brain

The determination of embolic effects on the brain also may be performed via perfusion scanning. PET or MRI, if available, can be used to measure brain perfusion in vivo, and radionuclide imaging also can be used with single photon emission computed tomography.^{14,15} Histopathology of the brain should be examined for plaques, evidence of amyloid, neurofibrillary tangles and neuropil threads, and angiopathy including hemorrhage.

Quantitative Histomorphometry

Morphometry of histopathologic sections is essential for stent evaluation. Measurement systems should be calibrated and documented against a traceable standard before each measurement session. Measurements of all sections should in-

TABLE 2. Important Safety Parameters to Be Evaluated in Animal Studies of Drug-Eluting Stents

Unexpected death and reasons
Clinical problems (death, fever, intolerance, and changes in gait, stance, or neurological status)
Stent thrombosis, infarctions, or both
Tissue ischemia, infarction; acute, healed, or both
Cellularity and description of drug-eluting vs control media
Medial area or presence or absence of medial thinning comparison
Inflammation at stent strut sites
Reaction to polymer or carrier
Endothelialization (scanning electron and light microscopy, functional assessments, if available)
Fibrin presence and degree
Angiographic and histopathologic stenoses

clude medial area, area within the internal elastic lamina, area within the external elastic lamina, lumen area, and stent area (area within the stent itself).

Neointimal measurement tests efficacy and should include thickness at each stent strut site and in between the struts, the mean of these values, and total neointimal area. The average neointimal thickness (average for all strut sites) should be calculated for each section. If the stent struts separate from the area within the internal elastic lamina, then each site should be measured and reported for distance of the strut separation.

Calculations Derived from Quantitative Histomorphometry

The following calculations from histopathologic information should be derived for each stent.

- Remodeling should be calculated for the mid-stent region as area within the external elastic lamina/area within the external elastic lamina proximal reference).
- Remodeling at the proximal and distal reference vessels should be calculated as reference area within the internal elastic lamina diameter/area within the internal elastic lamina diameter at midstent.
- Percent stenosis should be calculated 3 ways: (1) $100 \times (1 - \text{lumen area}/\text{area within the internal elastic lamina})$, (2) $100 \times (1 - \text{lumen area}/\text{proximal reference area})$, (3) $100 \times (1 - \text{lumen area}/\text{distal reference area})$.
- Peri-stent effects should be calculated as neointimal area proximal or distal reference – neointimal area mid-stent).

Statistical Analysis of Safety and Efficacy

Safety assessment should include the parameters shown in Table 2. Efficacy should be determined with a statistical comparison across the parameter groups shown in Table 3. Values obtained along the length of an artery do not represent individual statistical events and should be averaged and used as 1 data point per segment.

Report Summary and Conclusions

The conclusion section of the preclinical study should concisely state the motivation for the study design, justify the doses chosen, and enumerate the time points used. Conclu-

TABLE 3. Important Histopathologic Efficacy Parameters to Be Evaluated in Animal Studies of Drug-Eluting Stents

Lumen area
Percent stenosis
Neointimal area
Neointimal thickness
Medial thickness at stent strut sites
Medial thickness between stent struts
Remodeling
Injury
Inflammation
Radial gap width
Peri-stent segments
Overlap segments
Incomplete apposition

sions about dose- or exposure-related behavior should be supported by pharmacokinetic data. Proposed dose safety margins should be determined and justified by the data. Toxicities should be described as evident from the data.

Study conclusions based on a critical analysis of blinded data from an adequate number of animals studied are crucial for understanding device safety and efficacy. Conclusions should simply restate the data, but they should be an ordered interpretive list of stent safety, toxicity (including proposed toxicity margins), and efficacy. The appropriate use of representative graphics (charts, tables) should be included to support and simplify the conclusions.

Conclusions should be summarized and then synthesized. Conclusions should be drawn indicating whether the devices performed better than control (bare stent) and polymer or carrier-only devices (ie, relatively suppressed neointima formation and no excess neointima by histopathology). If such a statement is not possible because of mixed results, then the efficacy statement should be explicitly enumerated separately for all of the above parameters. Ideally, the drug-eluting device should perform better than either controls or the polymer or carrier-only devices. At a minimum, the performance of the drug-eluting devices should be no worse than the controls and polymer or carrier-only devices. If no efficacy difference is found in the preclinical morphometric studies between drug eluting versus bare and polymer-coated controls, then proceeding to human clinical trials may be considered from a risk:benefit perspective. This decision should be based on whether the device appears to be safe in preclinical studies after evaluating drug biological effects such as delayed healing. Finally, the report should reference whether the stented vessels healed completely, partially, or not at all.

Consensus Opinion: Satisfactory Findings and Outcomes

Scientific study results often are mixed, reflecting individual variations in response, and conclusions require interpretation. Study conclusions should reflect the success of a device under study and should not be interpreted as rigid requirements. At this early stage of peripheral model development, it

may not be possible to make quantitative recommendations for efficacy. The present writing group proposes the following general guidelines.

Stent Thrombosis

Experience with stented porcine coronary arteries suggests that sudden death may be more common in drug-eluting devices, principally because of platelet-rich coronary stent thrombosis.^{16,17} This problem is uncommon in peripheral stents, and thus local or embolic thrombotic events are not likely to be seen in peripheral stenting studies. Nevertheless, stent thrombosis should be considered in all cases of poor outcome. The early mortality rate for pigs should be $\approx 15\%$. Therefore, unexpected death occurring later than 24 hours after implantation should be vigorously investigated for cause. Overall mortality including early and late deaths should be $<25\%$, which is a high number. A study with such high mortality suggests that a problem exists with either the devices or the implantation methods and techniques. Any percentage of deaths higher than this number should be a warning of a substantial problem somewhere in the study.

Inflammation, Fibrin Deposits, and Fibrinoid Formation

Polymer coatings by their nature may induce fibrin deposits, inflammatory responses, and fibrinoid formation.¹⁸ Such responses may be acceptable if the reaction is minimal or mild, and the responses do not accelerate, extend, or cause substantial vascular injury or stenosis. It is crucial, however, that investigators demonstrate that later time points have inflammatory reactions that meet the same safety criteria as do early time points.

Neointima and Arterial Injury

Stent neointima should be thin, with adequate lumen at all time points. Deviations from these occurrences should be quantified and an assessment made by the pathologist who will determine whether such injury resulted from the drug- or polymer-associated inflammation or the mechanical injury of the stent itself. If conclusions of the study are made without including such severely injured sections, then the number and locations of such excluded sections should be stated.

Overall Conclusions

This document is intended to guide preclinical evaluation of drug-eluting stent technology for peripheral applications. It is a consensus opinion of active investigators and commercial entities in the field of interventional devices. It will be updated as needed and as more experience is gained with these new technologies. The judicious and wise use of these preclinical models may permit better understanding of the important relationships between these models and the clinical results in humans, and provide substantial improvements in evaluating these exciting devices. A more informed use of the animal models awaits more knowledge of the pathogenesis of stent-associated neointima formation in animals and humans. Also important is the optimal timing and duration of elution of prophylactic drugs from these devices. The reader is

referred to multiple references on models, stenting, assessment, and effects.^{8,9,19-39}

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References

- Schwartz RS, Edelman ER, Carter A, et al. Drug-eluting stents in pre-clinical studies: recommended evaluation from a consensus group. *Circulation*. 2002;106:1867-1873.
- Duda SH, Pusich B, Richter G, et al. Sirolimus-eluting stents for the treatment of obstructive superficial femoral artery disease: six-month results. *Circulation*. 2002;106:1505-1509.
- Deleted in proof.
- Deleted in proof.
- Deleted in proof.
- Huckle WR, Drag MD, Acker WR, et al. Effects of subtype-selective and balanced angiotensin II receptor antagonists in a porcine coronary artery model of vascular restenosis. *Circulation*. 1996;93:1009-1019.
- Virmani R, Guagliumi G, Farb A, et al. Localized hypersensitivity and late coronary thrombosis secondary to a sirolimus-eluting stent: should we be cautious? *Circulation*. 2004;109:701-705.
- Farb A, Burke AP, Kolodgie FD, et al. Pathological mechanisms of fatal late coronary stent thrombosis in humans. *Circulation*. 2003;108:1701-1706.
- Virmani R, Kolodgie FD, Farb A, et al. Drug eluting stents: are human and animal studies comparable? *Heart*. 2003;89:133-138.
- Deleted in proof.
- Pelisek J, Fuchs A, Engelmann MG, et al. Vascular endothelial growth factor response in porcine coronary and peripheral arteries using non-surgical occlusion model, local delivery, and liposome-mediated gene transfer. *Endothelium*. 2003;10:247-255.
- Meller J, Sahlmann CO, Becker W. Nuclear medicine studies in the dialysis patient. *Semin Dial*. 2002;15:269-276.
- Bennett HF, Li D. MR imaging of renal function. *Magn Reson Imaging Clin N Am*. 1997;5:107-126.
- Hyun Y, Lee JS, Rha JH, et al. Different uptake of ^{99m}Tc-ECD and ^{99m}Tc-HMPAO in the same brains: analysis by statistical parametric mapping. *Eur J Nucl Med*. 2001;28:191-197.
- Ding HJ, Huang YF, Tzeng CC, et al. Synthesis of Tc-D,D-HMPAO and Tc-L,L-HMPAO and their comparison of chemical and biological properties. *Bioorg Med Chem Lett*. 1999;9:3199-3202.
- Ahn YK, Jeong MH, Kim JW, et al. Preventive effects of the heparin-coated stent on restenosis in the porcine model. *Cathet Cardiovasc Interv*. 1999;48:324-330.
- Jeong MH, Owen WG, Staab ME, et al. Porcine model of stent thrombosis: platelets are the primary component of acute stent closure. *Cathet Cardiovasc Diagn*. 1996;38:38-43.
- van der Giessen W, Lincoff A, Schwartz, R, et al. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. *Circulation*. 1996;94:1690-1697.
- Janicki C, Hwang CW, Edelman ER. Dose model for stent-based delivery of a radioactive compound for the treatment of restenosis in coronary arteries. *Med Phys*. 2003;30:2622-2628.
- Welt FG, Tso C, Edelman ER, et al. Leukocyte recruitment and expression of chemokines following different forms of vascular injury. *Vasc Med*. 2003;8:1-7.
- Hwang CW, Wu D, Edelman ER. Impact of transport and drug properties on the local pharmacology of drug-eluting stents. *Int J Cardiovasc Intervent*. 2003;5:7-12.
- Hwang CW, Edelman ER. Arterial ultrastructure influences transport of locally delivered drugs. *Circ Res*. 2002;90:826-832.
- Lowe HC, Schwartz, RS, Mac Neill BD, et al. The porcine coronary model of in-stent restenosis: current status in the era of drug-eluting stents. *Catheter Cardiovasc Interv*. 2003;60:515-523.
- Rogers JH, Chang D, Lasala JM. Percutaneous repair of coronary artery bypass graft-related pseudoaneurysms using covered JOSTENTs. *J Invasive Cardiol*. 2003;15:533-535.
- Lepor NE, Rogers CD. Coronary artery disease treatment in the catheterization laboratory: the promise of an evolving new pharmacomechanical approach. Introduction. *Rev Cardiovasc Med*. 2002;3:S1-S3.
- Machan L, Burt HM, Hunter WL. Local delivery of chemotherapy: a supplement to existing cancer treatments. A case for surgical pastes and coated stents. *Adv Drug Deliv Rev*. 1997;26:199-207.
- Guagliumi G, Virmani R, Musumeci G, et al. Drug-eluting versus bare metal coronary stents: long-term human pathology. Findings from different coronary arteries in the same patient. *Ital Heart J*. 2003;4:713-720.
- Cheneau, E, John MC, Fournadjiev J, et al. Time course of stent endothelialization after intravascular radiation therapy in rabbit iliac arteries. *Circulation*. 2003;107:2153-2158.
- Guagliumi G, Farb A, Musumeci G, et al. Images in cardiovascular medicine. Sirolimus-eluting stent implanted in human coronary artery for 16 months: pathological findings. *Circulation*. 2003;107:1340-1341.
- Finn AV, Gold HK, Tang A, et al. A novel rat model of carotid artery stenting for the understanding of restenosis in metabolic diseases. *J Vasc Res*. 2002;39:414-425.
- Hiatt BL, Carter AJ, Yeung AC. The drug-eluting stent: is it the Holy Grail? *Rev Cardiovasc Med*. 2001;2:190-196.
- Carter AJ. Drug-eluting stents for the prevention of restenosis: standing the test of time. *Catheter Cardiovasc Interv*. 2002;57:69-71.
- Serruys PW, Regar E, Carter AJ. Rapamycin eluting stent: the onset of a new era in interventional cardiology. *Heart*. 2002;87:305-307.
- Suzuki T, Kopia G, Hayashi S, et al. Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. *Circulation*. 2001;104:1188-1193.
- Farb A, Heller PF, Shroff S, et al. Pathological analysis of local delivery of paclitaxel via a polymer-coated stent. *Circulation*. 2001;104:473-479.
- Virmani R, Farb A, Carter AJ, et al. Pathology of radiation-induced coronary artery disease in human and pig. *Cardiovasc Radiat Med*. 1999;1:98-101.
- Klugherz BD, Llanos G, Lieuallen W, et al. Twenty-eight-day efficacy and pharmacokinetics of the sirolimus-eluting stent. *Coron Artery Dis*. 2002;13:183-188.
- Klugherz BD, Song C, DeFelice S, et al. Gene delivery to pig coronary arteries from stents carrying antibody-tethered adenovirus. *Hum Gene Ther*. 2002;13:443-454.
- Nasser TK, Mohler ER III, Wilensky RL, et al. Peripheral vascular complications following coronary interventional procedures. *Clin Cardiol*. 1995;18:609-614.

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