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## Drug-Eluting Stents in Preclinical Studies Updated Consensus Recommendations for Preclinical Evaluation

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### Abstract

Coronary drug-eluting stents are commonplace in clinical practice with acceptable safety and efficacy. Preclinical evaluation of novel drug-eluting stent technologies has great importance for understanding safety and possibly efficacy of these technologies, and well-defined preclinical testing methods clearly benefit multiple communities within the developmental, testing, and clinical evaluation chain. An earlier consensus publication enjoyed widespread adoption but is in need of updating. This publication is an update, presenting an integrated view for testing drug-eluting technologies in preclinical models, including novel devices such as bioabsorbable coatings, totally bioabsorbable stents, bifurcation stents, and stent-free balloon-based drug delivery. This consensus document was produced by preclinical and translational scientists and investigators engaged in interventional technology community. The United States Food and Drug Administration (USFDA) recently issued a Draft Guidance for Industry Document for Drug-Eluting Stents. This expert consensus document is consistent with the Food and Drug Administration guidance. The dynamic nature of this field mandates future modifications and additions that will be added over time.

### Keywords

drugs; restenosis; stents

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Drug-eluting stents (DES) are commonplace in clinical practice, and show acceptable safety and efficacy clinical profiles. Safety of these devices appears predictable from preclinical models, though DES efficacy may be difficult to establish from such studies. As these devices evolve in complexity, correlative clinical data has accumulated, and the importance of preclinical models continues to grow. It is clear that well-defined preclinical testing methods

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benefit the Interventional Medical, Regulatory, Scientific, and Industry communities. These guidelines are intended for preclinical investigators in academics, independent testing laboratories, and for companies designing and performing preclinical DES studies. It is also hoped that the guidelines will be useful for regulators and regulatory agencies.

An earlier publication from our consensus group served as a standard, but this document needs updating. This consensus is an updated and integrated view of requirements for evaluating drug-eluting technology in preclinical models. It includes novel technology such as bioabsorbable coatings, totally bioabsorbable stents, and stent-free balloon-based drug delivery. As mentioned earlier, requirements encompass study design, experimental performance, and histopathologic evaluation, emphasizing safety across multiple time points. We also include suggestions for more sophisticated investigations that can provide structural and functional data specifically designed to address problems recently identified with current DES.

The United States Food and Drug Administration (USFDA) has recently issued a draft guidance for industry document for drug-eluting stents. This consensus document is consistent with, and complementary to, this proposed guidance. Table 1 outlines principal similarities and differences between the 2 documents.

This consensus was produced by preclinical and translational scientists and investigators engaged in interventional device evaluation and development. The suggestions are a loose “standard,” but do not prescribe a single method for all technologies to be evaluated. The methods herein motivate evaluation and indicate how evaluation should be performed. It is understood that methods will change and knowledge will evolve, in particular as correlation is established with clinical data. The dynamic nature of this document allows for future modifications and additions.

## Definition

A DES is an intravascular device that mechanically supports a blood vessel and presents or releases single or multiple bioactive agents to cells that interact with the stent. Nonstent-based drug delivery comprises the same fundamentals, but without a stent or implanted device. The bioactive drug or agent(s) may be eluted or injected into the vessel wall and bloodstream, or may be bonded on the device surface. This bioactive substance may be adherent or released from a modified metal surface, from a biostable or biodegradable strut or spanning component such as a polymer or coating. It may also be from a nonpolymeric mixture containing bioactive particles such as microspheres.

## Overview

The following general principles apply to standardization. Pharmaceutical agents or other bioactive components intended for DES formulations or catheter-based agent delivery undergoing preclinical evaluation should have sound theoretical and practical reasons to anticipate biological success, namely producing a favorable balance between neointimal growth, endothelial cell coverage, vessel remodeling, and thrombus deposition. It is preferable that at a minimum, affirmative *in vitro* data exist for the bioactive substance(s) at the *in vitro* or cell culture level, with specific cellular targets.

The bioactive component can deposit in and affect blood vessels, cells, plaque, and tissues adjacent to the stent or at a distance from the stent. Systemic drug exposure should be controlled within a biologically safe concentration given the known effects of the compound. In some cases, systemic drug elution may be desirable given the biological effects of the compound and therapeutic target. Drug can be embedded and released from within (matrix-type) or

surrounded by and released through (reservoir-type) carrier substances (typically but not necessarily a polymer) that coat (strut-adherent) or span (strut-spanning) the struts of the DES. The carrier may be biodegradable or biostable (not absorbable). It may be only a thin coating on the stent, or it may be a compound released from a delivery technology. The configuration may be a balloon that actively delivers drug, drug plus polymeric material carrier, or bioactive agent directly to the arterial wall, without a stent. This includes needle-based technologies that inject directly into the artery wall. In other formulations, the drug may be linked to the stent surface without the need for a coating by means of detachable bonds that release with time, removed by active mechanical or chemical processes, or in a permanently immobilized form that presents drug to flowing blood. The stent platform may be a simple modification of clinically available devices or units specially designed for drug elution.

## In Vitro and In Vivo Pharmacokinetics

Drug or bioactive substance release from the proposed surface should be characterized both in vitro and in vivo. The former is a valuable assay of manufacturing quality control and the latter of potential functionality. In vitro release should be examined at body temperature, under “infinite-sink” conditions, and with agitation to prevent boundary layer effects until completion of release or no significant change in release is further anticipated. This should be performed in appropriate solvents or detergents as determined by the physicochemical properties of the agent. Accordingly, it is advantageous to define these agent-related properties including, for example, diffusivity in free water and in the optimal solvent, solubility, oil:water partition coefficient, degree of protein binding, and molecular weight and charge. For example, the release of protein binding drugs should be examined in protein-containing solutions. Elution features may differ across release platforms as well, and kinetics should be presented from the devices to be implanted. In contrast to release profiles from surface-bound or those incorporated within nondegradable materials, release from degradable coatings may cease after a time and then resume, and these should be described.

In vivo release kinetics may be characterized in a number of ways. Direct chemical determination of release or presence of radio- or fluorescent-labeled compounds in serum can be used to construct release curves. Agents with first pass metabolism might be detected in urine. Alternatively or additionally, stents can be recovered at variable points in time after implantation and the amount of residual drug determined and used to extrapolate a release kinetic. When first-order release kinetics are observed, a release half-life ( $t_{1/2}$ ) should be determined. Some investigators suggest that a loose definition of half-life can be helpful for all release formulations with this parameter defined as the time at which half of the drug has left the stent. The sensitivity of characterization is maximized by frequent sampling. A minimum of 5 time points examining release kinetics from 3 separate devices is recommended, even in devices that are not intended to release, in which case documentation of stability should be reported. The in vivo studies should enable construction of terminal elimination equation and tissue samples obtained to validate the modeling.

Concentration in blood, in the coronary artery wall at the immediate implant site, and in myocardium directly beneath the stent or delivery site (if no stent is used) should be measured at multiple time points. These times should cover the range of elution from immediately after implantation until the time when most drug is eluted. Drug concentration should also be measured in downstream myocardium supplied by the target artery segment in short term, acute (hours–days) studies. Long-term drug concentration in liver, kidney, and lung at necropsy should also be measured to verify whether systemic effects are possible.

Several additional, optional measurements are desirable for in vivo pharmacokinetics estimation. These include 1) additional time points to more fully characterize drug release into

arterial tissue and better definition of safety margin dose, 2) drug levels in arterial tissue proximal and distal to the device, and 3) drug levels in myocardial tissue proximal and distal to stent. Table 2 summarizes the pharmacokinetic recommendations.

Drug kinetic testing in normal tissue versus diseased tissue is an important question. At this time, normal tissue is perhaps best to understand tissue kinetics, though diseased tissue models may provide a more accurate environment to understand drug levels, retention, and washout in diseased human vessels. Nonstent-based devices such as drug-eluting balloons should be considered for closer time points and shorter follow-up periods given early data from such technologies that suggest more rapid disappearance kinetics.

Multidrug-eluting stents should be tested with both in vivo and in vitro. In vivo tests should be performed for each drug separately and for the combination together to determine hypothetical drug interactions. This is neither practical nor efficient to test in living animal models. Therefore, the composite device alone can be tested in vivo, although assays for each agent should be carried out and compared with in vitro results. This should be done at all time points to establish aberrations that may occur in the preclinical model environment.

## Dose

The proposed dose and kinetic release characteristics planned for clinical application should be justified by preclinical data. Preclinical dose ranging is strongly recommended, showing biological effects across dose ranges from subtherapeutic to toxic levels. Such a toxic high-dose value could be, for example, within the range 3 to 10 times the anticipated clinical dose. This high dose will also be useful in estimating a safety margin, which is very desirable. However, it is recognized that many local therapies do not exhibit a dose response for efficacy, only for toxicity.

Dose finding helps define toxicity and should include the highest possible dose that can be loaded on a stent or released at any point in time from a balloon or device. A dose representing a safety margin should be estimated and justified by the spectrum of biological responses in the multiple dosing studies. The dose at which toxicity appears should be documented by histopathology and possibly correlated with cell culture studies. A safety margin dose is one in which toxic effects are beginning to appear, or one in which higher doses show clear evidence of toxicity. This dose can later be used to justify safety for a clinically chosen dose.

Ideally, a multiple-dose study should thus be performed in an acceptable animal model to establish safety margins, efficacy, and toxicity in choosing a dose for clinical trials. This may be difficult to perform because the maximum concentration of agent that can be loaded on the device platform may not be substantially greater than that is anticipated to minimally effective. When possible, results obtained with the maximal possible loading of drug should be presented along with lower doses. Ex vivo studies with the test device should be undertaken to understand the total dose that will be delivered.

## Stent and Drug Release in Cell Culture Studies

It is accepted that biological effects seen in cell culture do not necessarily correlate with in vivo activity. However, if the biological effects of the agent to be eluted are examined in cell culture, such experiments should use vascular endothelial and smooth muscle cells over a range of doses in a logarithmic scale. Although human cells are preferable, they may be less practical, so that porcine or rabbit cells will suffice.

## Animal Models

The ideal animal model for DES evaluation remains uncertain although several excellent models have emerged. Drug deposition and in vivo pharmacological response will vary with vascular site and local lesion morphology. It is unclear that any single animal species is more indicative of the potential human clinical response and for the indications desired. As such, animal models provide mechanistic insight into fundamental biological processes and appear at a minimum to indicate relative safety. Furthermore, preclinical models allow testing critical hypotheses regarding putative mechanism of action of an intervention.

There is no perfect animal model of human vascular disease. Research into correlative data between animal models and human clinical application is underway in hopes of predicting therapeutic features of safety, efficacy, and practicality in reliable animal models. Proof of concept can be examined in animals including evidence for toxicity based on histopathologic effects and advanced cell/tissue analytic techniques. True efficacy and safety can currently only be proven in humans, so it is critical to construct human trials that resemble the animal preclinical trials and to make it clear what data and important conclusions can be justifiably extracted from animal models.

Experience suggests that the coronary arteries in domestic crossbred or miniswine, or rabbit iliac arteries are suitable because the size, access, and injury response appear similar to human vessels, and therefore may permit device evaluation before clinical evaluation. Selection of devices with comparable and proper dimension is essential. Stents should be appropriately sized to the target vessel for preclinical studies, because the mechanics of stent placement often play key roles in vascular responses for safety and efficacy. Intravascular ultrasound (IVUS) or optical coherence tomography (OCT) guidance is an excellent method to accurately size the vessels.

Safety and efficacy should be examined in a comparative study with multiple time points. One important safety concern is vessel thrombosis. All animals experiencing death or other untoward clinical events should be examined and the treated vessel status carefully documented, regardless of cause of death. The overall health status of the animals should be documented on a daily basis by the laboratory staff under the direction of a veterinarian or qualified study director. Specifically, activity level, dietary intake, and major organ systems (skin, respiratory, cardiac, and gastrointestinal) should be regularly examined. Other laboratory parameters may be appropriate to monitor at intake and on conclusion of the study given the known biological effects of the compound.

Standard stent practice in patients entails oral aspirin plus either clopidogrel or ticlopidine, or other agent intended for clinical use. These agents should be administered throughout the preclinical study as planned for clinical use. In general, antiplatelet agent use should be dictated by questions to be answered and should follow clinical standards unless specifically indicated.

Antithrombotic stents should be considered for evaluation as follows. Indium-111 labeling may be performed to establish thrombus mass. However, Indium-labeling and assessment is technically challenging, often with substantial variability and poor correlation with clinical events. A simpler method for thrombogenicity testing is an animal model without clopidogrel antiplatelet medication, for example, aspirin only. If efficacious, an antithrombotic stent will collect thrombus but not completely thrombose. Such stents should be removed within 1 hour of implant, thrombus stripped from the device, and the thrombus mass determined by weight.

## Models, Pharmacokinetics, and Tissue Response

### Porcine Coronary Artery Model

The porcine model of choice is the normolipemic domestic crossbred or miniswine coronary artery. The arteries of domestic swine grow substantially over time, and this fact must be considered for long-term testing in pigs. Stents or drug-delivery balloons should be appropriately sized for the target vessel (device:artery ratio between 1.0 and 1.2) and implanted into naïve arteries with no prior injury. Double injury models are biologically interesting but vary more widely in biological response, thus affecting reliability and reproducibility of the evaluation. Vessel wall injury is best quantified by light microscopy using well-validated methodology to derive an ordinal measure or injury score (Table 3).

Peripheral porcine arteries are more elastic, are less prone to overstretch injury, do not develop neointima as vigorously as coronary arteries, and are therefore less desirable for testing DES intended for coronary application. However, peripheral artery testing may be a good model for DESs or local drug therapy intended for peripheral use.

The normal coronary arteries of juvenile normolipemic pigs endothelialize more rapidly following injury juvenile pigs, typically within 4 weeks. If confluent endothelium is present within 28 days of treatment or stent placement, this finding does not necessarily assure favorable human endothelial reaction. However, if the porcine model suggests substantial or long-term endothelial toxicity as manifested by incomplete endothelialization of the stent, this observation suggests but does not prove potential problems with vascular biological compatibility. Diseased porcine animal models are undergoing extensive validation and may potentially provide closer biological responses compared with the juvenile healthy porcine model but cannot be yet recommended.

### Rabbit Iliac Artery Model

The rabbit iliac model is an accepted and validated method to assess feasibility, safety, and biocompatibility of DES. Although clearly useful, it is not a mandatory component to device testing. Naïve normolipemic, hypercholesterolemic, and atherosclerotic models have been used for preclinical stent and DES studies. As with the porcine model, stents or treatment balloons should be appropriately sized (treatment:vessel ratio between 1.0 and 1.1) and implanted into vessels without prior injury. This model is suboptimal for survival endpoints designed to monitor thrombotic or other clinical complications because subacute thrombosis or arrhythmias originating in downstream myocardium due to the drug or treatment are not detectable in peripheral implants.

### Drug-Eluting/Bioactive Stents and Controls

Only one test formulation stent or catheter-based treatment should be used in an artery except when study objective or scientific hypothesis necessitates treatment overlap or multiple dosing data. DES formulation, stents or other catheter-based therapies may be placed in multiple different arteries in the same animal. In general, most study designs incorporate a test formulation with either a bare or carrier-only stent implanted in 2 or 3 major epicardial arteries as appropriate controls.

The design of a scientifically valid preclinical study must incorporate appropriate controls to ascertain specific treatment effects or the screen for toxicity. Ideally, the study design will incorporate test and control articles enabling biocompatibility and safety endpoints for each of the critical components (ie, stent, polymer, drug, and polymer formulation). The design of such studies should be undertaken with a clear understanding of material properties and anticipated biological behavior. Polymeric or other carrier materials for drug elution frequently affect the



vascular response and arterial repair, generally in an undesirable manner. When drug or other agent is bound directly to a stent, the stent or therapy without drug can be a satisfactory control. However, when a polymer or carrier of any sort is present, additional controls to evaluate the carrier alone, without drug, must also be included. Coatings of polymer or carrier materials and not loaded with drug will react differently than coatings devoid of drug or agent after complete release. This may reflect a difference in surface characteristic (eg, porosity, texture, etc.) especially when matrix-type devices are used. Care must be taken not to include a treatment arm that will likely induce potentially fatal complications and it should be remembered that likelihood of animal death rises with number of interventions.

## Overlapping Stents or Treatments

Stent overlap occurs often in clinical implants (30% or more of coronary percutaneous coronary intervention [PCI] cases), and overlapping treatments present the possibility of additive or synergistic effects from drug released from the 2 overlapped sites. Preclinical studies for DES should be conducted in single and overlap models. The initial proof of concept, safety, and biocompatibility testing may be conducted in single stent models. Stent overlap is also good to evaluate stent fracture as it provides a hinge point for the distal stent.

Although avoiding overlap during initial evaluation, purposeful overlap should be performed in later studies to determine safety interactions. The distance of overlap should be roughly one third the length of a stent or a minimum of 4 mm, and the number of overlapping stent implant pairs should be no less than 8. Histopathologic analysis should include sections taken from the nonstented reference segments, the single (nonoverlapped) treatment region, and from the overlapping region.

## Stent Fracture

Stent fracture represents an undesirable mechanical failure of the prosthesis that may introduce further vessel wall injury, potentiate an inflammatory or thrombotic response, and corrupt drug delivery. Preclinical device studies should incorporate accepted methods to screen for acquired strut fracture. This typically involves a high resolution magnified x-ray from several views that adequately assess the possibility or severity of stent fracture and tissue effects. In vivo or ex vivo computed tomographic angiography (CTA) or rotational angiography should be considered if available. The fracture site should be sought specifically for histopathologic study for biological effects and compared with sites without fracture. The frequency, pattern, and stent fracture type (single or overlapping stents) and observed biological effects should be reported in all preclinical studies.

## Sampling Time Points and Sample Size

Safety should be assessed by clinical and histopathologic endpoints such as sudden cardiac death due to thrombosis (defined as sudden unexpected death after recovery from anesthesia, and confirmed by gross observation or light microscopy), inflammatory response (cell type, extent, temporal features), and generally by neointimal response over time. The mean neointimal area should be similar to or less than bare metal control stents at all prespecified time points. Clinical and histopathologic data should be obtained at an early time point (3 to 7 days) to help determine subacute thrombosis risk by comparison of mean thrombus/neointimal area for DES test formulation and bare metal control stents. Short-term endpoints are desirable if endothelialization rates are the objective of the study. Typically, less than 14 days for the porcine model and 28 days for the rabbit model are satisfactory.

Experience has shown that the 28-day time point yields important healing information. At 90 days, differences between technologies may emerge, and thus, these 2 time points are essential.

The 28-day point is also important to quantitate neointimal hyperplasia. At least 2 (or more) late time points (eg, 180 and 360 days) should also be tested to examine long-term effects, specifically neointimal hyperplasia, and healing as assessed by endothelial coverage, residual fibrin deposition, calcification, inflammation and granuloma formation, and qualitative vascular cellularity. Drug is typically cleared within 4 half-lives when eluted from passive strut-based surface coatings, thus, providing a theoretical time construct for determining the duration of late study endpoints. In cases of DES formulations with slow-release profiles, late studies are particularly important for documenting safety. All DES preclinical study programs should include a very late time point, 360 days or longer, based on pharmacokinetics and documented preliminary biological effects of the DES formulation to characterize the vascular response after the terminal drug elution time.

Three-month follow-up is usually acceptable for initiating investigational device exemption (IDE) clinical trials if no adverse findings are noted at this time and if vascular responses are no worse than earlier time points. However, longer term data should be pending at the time of IDE submission. These later time points are especially important given the impact of persistent late remodeling, as an additional cause of persistent effects that would impact the late clinical outcome. Late remodeling should be aggressively sought and documented, especially as it relates to regions where the vessel wall may have remodeled to the point of losing contact with the stent struts.

It should be noted that long-term time points have been traditionally used for safety and biocompatibility assessment and remain insufficiently validated for efficacy endpoints. Nevertheless, it is desirable to document a sustained treatment effect or at a minimum biological equivalence for DES formulations in comparison with bare metal stents after 28-days in preclinical models. To date, a comparison of published long-term studies with selected DES formulations suggest that late vascular response (ie, neointimal formation after 28 days) may or may not correlate with clinical restenosis or increased target lesion revascularization (TLR) in clinical trials out to 4 years. The importance of determining healing parameters cannot be overstated, especially in relation to bioactivity release of the agent. Tissue reactivity and general animal health should be examined for a multiple of stent residence or treatment times within the primary target tissue.

## Special Consideration

### Totally Bioabsorbable Stents and Absorbable Coatings

The advent of bioabsorbable polymers and stents for vascular applications mandates special consideration. Completely absorbable stents should be evaluated at a minimum in a manner similar to the above guidelines for biostable stents. In vivo dissolution chemistry (whether based on polymers, metals, ceramics, biomaterials, or other completely absorbable materials) should be documented by appropriately designed experiments with sufficient temporal duration beyond material degradation. Histopathologic endpoints and material degradation studies may be designed to determine in vivo material transformation, dissolution, half-life, and vascular response. The local vascular tissue response and systemic toxicity should be carefully investigated and documented, as should absorption time. It is recommended that histopathology be available for more than 3 to 5 time points in the dissolution process: 1) early: 24 to 48 hours for very early assessment of mechanical features and then within 7 days of implant, 2) late: after total dissolution, 3) intermediate: using 3 additional, justifiable, equally interspersed time points. These recommended sampling intervals are intended to serve only as an example for design of experiments sufficient to document in vivo material degradation and long-term histopathologic response. The constructs may apply for strut-based bioabsorbable coatings on metallic stents as well as a fully bioabsorbable stent and coating. In the event that material degradation is very long (greater than 2 years), it may be acceptable to use a 12-month



time point to determine safety as defined elsewhere in this document. In this case, clinical trials should be conducted with sufficient longitudinal follow-up to determine safety.

Alternative noninvasive and serial invasive imaging studies may be conducted to assess safety, biocompatibility, and vascular function. These data may be particularly useful at intermediate time points and enable more efficient use of research animals. Cardiac computed tomography angiography, coronary angiography, vasomotor studies, IVUS, or OCT can be used to document several integral aspects of vascular repair and function over time to complement histopathologic end points. Assessment immediately after implant is important to assess immediate recoil. Late recoil should also be assessed, and expressed over time, especially for bioabsorbable technologies.

These in vivo methods may be particularly important to document specific biological effects such as restoration of coronary vasomotor function and vascular remodeling that may not be sufficiently determined by present histopathologic techniques. This will function to document safe absorption with favorable histopathologic results and trajectory and stability of vascular reparative effects. Bioabsorbable coatings on biostable stents should be evaluated the same as totally bioabsorbable stents, with interim time point evaluations.

### **Bifurcation Stents**

Recently, several bare metal and drug-eluting stents are under evaluation for bifurcation and ostial lesions. Evaluation of these stent systems poses additional challenges as they often have unique shapes reflecting the peculiar anatomy they are intended to treat and/or may consist of several components addressing the main vessel and the side branch. As such, they may involve overlap of 2 or more investigational devices, or of the investigational device with an approved stent. Consequently, their testing necessitates adequate anatomic models (eg, sheep coronary system with its more frequent human-sized bifurcations) and increases complexity of outcome analysis in the preclinical setting.

### **Number of Implanted Stents or Treatment Sites**

The number of vascular treatment sites should be determined from a power calculation for predetermined expected difference in key parameters. Sample size power calculations are not well defined, and must so be estimated. Typically, 10 to 12 test DES formulations per time point are satisfactory in most models.

### **Implant Procedure or Treatment**

Veterinary anesthesia should be established per accepted standard per American Veterinary Medical Association (AVMA), in compliance with local Institutional Animal Care and Use Committee (IACUC) and Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) standards. Surgical technique for the procedures including vascular access sites, catheters, wires, and other procedural equipment may be at investigator discretion but must be in compliance with published studies and compatible with current clinical practice standards.

### **Stent-Related Antiplatelet Medication**

All animals should receive antiplatelet therapy (aspirin plus clopidogrel or ticlopidine) daily, beginning with a loading dose 1 day before the procedure and continuing for the survival duration. This should be considered especially for bifurcated stents or when multiple devices are implanted in a single animal.

## Autopsy/Necropsy Evaluation

Comprehensive necropsy is an important part of the evaluation process and validity of the test model. All premature and unexpected deaths should be closely examined by necropsy, gross evaluation, and histopathologic examination. Special attention should be given to the treatment and control sites as possible causes. The term “procedural death” should be avoided or carefully explained and documented, recognizing that death after the first 24 hours both in pigs and in rabbits is rare and may represent problems with the treatments under test. Thrombus at treated sites or within stents should undergo histopathologic examination, and determined to be either premortem or postmortem. In general, a variegated platelet-fibrin component, clot layering, clot adhesion to the vessel wall, and polymorphonuclear leukocytes with cellular organization and maturation suggest premortem stent thrombosis. These features should be detailed in the study report.

Necropsy should be performed by a qualified individual to determine the cause of death for all animals dying following entry into the study, regardless of whether completing the allotted survival time or not. An opinion should always be rendered as to cause of death when not due to euthanasia. The status of all stents or treatments in such early or unexpected deaths should be determined, recoded, and reported. The heart should be examined for any evidence of myocardial infarct or fibrosis especially in the vascular distribution and perivascular region (respectively) of the treatment.

Histopathology should be performed on all treatment sites, including those in animal dying any time after initiation of the procedure. All device sites 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 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 should be performed at about 100 mm Hg and with rapid exsanguination. Immersion fixation should be performed in a volume sufficiently large to allow complete and rapid fixative percolation through the tissue and without alteration of the tissue shape. After removal, the hearts or vessels containing the stent should be sectioned transaxially (short-axis sections) at a minimum of 1-cm intervals. These sections should be examined grossly for evidence of myocardial 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 treatment performance and effects, either positive or negative. Plastic or epoxy embedding is strongly recommended for metallic or hard devices, as paraffin sectioning with strut removal disturbs tissue and cell relationships. Diamond knife sectioning and grinding methods are both acceptable though diamond knife methods yield thinner sections and thus better cellular detail resolution.

Gross tissue effects can be visualized with hematoxylin and eosin, elastin stains, and trichrome (preferably Masson) stain alone. More specific cellular responses require specialty stains and immunohistochemical techniques. A representative number of sections should be taken to examine the entire stent or treatment site, proximal and distal segments, and adjacent/affected tissues.

Immunostaining for cellular proliferation should be performed over the life of drug or agent release, or over the course of device dissolution in the case of completely absorbable devices. Proliferation should be estimated with standard methods such as bromodeoxyuridine, proliferating cell nuclear antigen, or Ki-67 immunohistochemistry.

A pathologist or other individual with extensive and specialized experience in microscopic examination of treated arteries must be the primary reviewer of tissue and treatments 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, mid, distal, and distal reference artery (minimum 10 mm).

## **“Clinical” and Blood Parameter Evaluation**

Animal well-being is an important observation after stent implant. Clinical features include normal physical signs and blood parameter measurements. The DES or treatment should have minimal effect on physical signs and clinical parameters in any animal implanted with a DES or undergoing local vascular drug treatment. 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. Evidence of myocardial infarction should be sought in the case of porcine coronary implant by performing electrocardiography at baseline compared with euthanasia.

Blood parameters should be measured to observe for allergic effects, liver, or renal dysfunction. These measures should be done at baseline and at euthanasia, and include a complete blood count with differential, liver enzymes (ALT, AST), and creatinine. Particular drugs may have idiosyncratic effects on tissues and cells and specific chemical parameters may need to be assayed in those instances.

## **Arteriography, IVUS, OCT**

Key to understanding device performance and compatibility is healing. Healing should be evaluated by stent coverage to the degree possible by imaging methods such as arteriography, IVUS, and OCT. Assessment of vessel coverage by the device at late time points should be assessed and stated explicitly. Late loss is a related parameter and should also be assessed. Arteriography immediately before euthanasia can yield important information about the arterial lumen and patency within the stent and should thus be performed. Special attention should be for arteriographic persistent effects. IVUS or OCT may be performed in a minority of stents to detect persistent effects and neointimal formation. Persistent effects typically are defined as including the 5 mm beyond the stent ends. However, routine IVUS or OCT in all animals may risk damaging the stented or treated vessel and should be considered only after careful thought.

## **Stent or Treatment Evaluation**

Simple visual description of the histopathology is discouraged as the sole evaluation. A more rigorous, semiquantitative and defined scale for device evaluation should also be presented. Additionally, OCT intravascular imaging appears useful for serial analysis of completely absorbable stent devices in vivo, for reasons of enhanced resolution as well as signal penetration through the implant. It can thus be used as one index to assess the rate of absorbable device erosion.

## Semiquantitative Histopathology

**Injury and Inflammation**—Inflammation by histopathologic evaluation should include injury assessment by a means of the Injury Score (value 0 to 3) at each stent strut site or for each section, an inflammation description (absent, cell types, location), an inflammation score (0–3), and endothelial characterization (see below) for the overall vessel as well as the adventitial, media, neointima, and at stent strut sites. When possible cell density in tissue compartments should be recorded as number of cells per area to document “drug effects.” It is recognized that granulomata occur regularly in stent studies. Exclusion from analysis of vessels containing granuloma is permissible, but the count of such vessels should be noted, and is typically less than 10% of vessels in preclinical studies.

**Angiogenesis and Other Histopathology**—Angiogenesis can also be scored (0–3) and reported for adventitia, media, and neointima. Other histopathologic features should be observed in the media, adventitial, and neointima, and assigned a value of 0 through 3 or a more quantitative parametric value such as number and size of vessels per unit area. These include fibrin or fibrinoid deposits, hemorrhage, and necrosis.

## Observational Histopathologic Data

**Endothelialization, Neointima, and Vessel Healing**—Endothelium restoration and neointimal coverage are markers of vascular repair in the animal and not of regenerative potential in the human. The physical presence of cells does not correlate with restoration of endothelial or normal vessel function, and that rates of endothelialization differ within each vascular bed and for different species. As such it is important to choose an arterial bed, animal species, and time point that provide a dynamic range if endothelial recovery is to be followed as a primary end point.

A healed vessel should show endothelialization or a healthy appearing layer of near-complete periluminal cells. Endothelialization should be recorded as absent, partial, or complete in all sections. Semiquantitative analysis can be performed and presented as the percentage of circumference covered by endothelium. The time of re-endothelialization should be estimated. Scanning electron microscopy from 3 or more stents is recommended to assess endothelial recovery. Careful expert consensus consideration of a “completely healed” vascular site also suggests that it demonstrates no evidence of fibrin, fibrinoid deposits, excessive inflammation, or hemorrhage.

**Stent Strut Position and Adjacent Tissue**—Other observational data should include device/stent strut apposition to the vessel wall (percent of wires in contact), and struts covered by tissue or endothelium (percent). A subjective description should also be rendered for adjacent tissue, including medial thinning, loss of cellularity, and hyalinization.

## Myocardial Histopathology

Histopathology from the myocardium directly beneath, distal to, and region supplied by the stent should be observed and recorded as normal or abnormal, and same or different from control stents. Specific attention should be directed at examination for myocardial infarcts.

## Quantitative Histomorphometry

Histomorphometry of histopathologic sections is essential for therapeutic evaluation. Measurement systems should be calibrated before each measurement session against a traceable standard. Measurements at all sections should include medial area, area within the internal elastic lamina (IEL area), area within the external elastic lamina (EEL area), lumen

area, and stent area (area within the stent itself). Late loss can be calculated as the known nominal (or measured) diameter at implant minus the loss parameter at euthanasia.

Neointimal measurement is important for efficacy assessment and should include thickness or area percent stenosis at each stent strut site and total neointimal area. The average neointimal thickness or area percent stenosis (average for all strut sites) should be calculated for each section. If there is separation of the stent struts from the IEL, each site should be measured and reported for distance of strut separation.

### Derived Calculations From Quantitative Histomorphometry

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

Remodeling should be calculated for the midstent region: (EEL area/EEL area proximal reference)

Remodeling at the proximal and distal reference vessels should be calculated: (Reference IEL diameter/(IEL diameter at midstent)) Percent stenosis should be calculated 3 ways:

1.  $100 \times (1 - \text{lumen area/IEL area})$
2.  $100 \times (1 - \text{lumen area/proximal reference area})$
3.  $100 \times (1 - \text{lumen area/distal reference area})$

“Persistent effects” should be calculated as (Neointimal area proximal reference – neointimal area midstent)

### Statistical Comparisons for Safety and Efficacy

Statistical analysis should be performed when possible. Continuous variables should be presented as mean±standard deviation and assessed with Student *t* test, or analysis of variance and correction for multiple comparisons. Dichotomous variables such as injury score, endothelialization, and inflammation cannot be averaged but should be presented categorically. Data analysis should include safety by specifically enumerating the parameters shown in Table 4. Efficacy should be quantitatively analyzed with a statistical comparison across groups for the parameters shown in Table 5. Values obtained along the length of an artery do not represent individual statistical events and should be averaged and used as one data point per segment.

### Report Summary and Conclusions

The study report conclusion section should begin with a concise segment stating motivation for the study design, rationale for the doses chosen, and for the time points used. These should be supported by pharmacokinetic data. Proposed safety margins should be determined and justified by the data. Toxicities should be described as evident from the data.

Study conclusions are crucial to understanding device safety and efficacy. These should be communicated clearly and concisely. Conclusions should not be simple data restatement but should be an ordered, interpretive list reflecting stent safety, toxicity (including proposed toxicity margins), and efficacy. Each conclusion should reflect synthetic thought, well supported by study data. Appropriate use of representative graphics (charts, tables, etc) should be included to support and simplify the conclusions.

The report should first summarize, and then synthesize conclusions. A general statement should be made based on study data indicating whether the drug-eluting devices performed better than control and polymer/carrier-only devices. If such a statement is not possible in broad terms

due to mixed results, the efficacy statement should be explicitly enumerated for all of the above parameters separately. Ideally, the drug-eluting device should perform better than controls and polymer/carrier-only devices. At a minimum, the drug-eluting devices should not be worse than controls and polymer/carrier-only devices. The report should reference whether the treated vessels healed completely, partially, or not at all.

## **Consensus Opinion: Satisfactory Findings and Outcomes**

It is well recognized that scientific study results are frequently mixed, and that conclusions require interpretation. The study conclusions should reflect general success of a given device study and should not be interpreted as rigid requirements. Comments should be made regarding quantitative neointimal results. General guidelines are mentioned below.

### **Sudden Death**

Experience with stented porcine coronary arteries suggests that sudden thrombosis is principally due to platelet-rich clot. Although such deaths typically occur in the first 24 hours after implant, they may occur later if healing is impaired. The early mortality rate for pigs should be less than about 10%, a number representing good technique and good stent technology. This is typically reduced when pigs receive 1 or 2 stents instead of 3. Sudden death later than 24 hours should be vigorously investigated for cause. A study with more than 10% spontaneous mortality suggests a problem with either the devices or the implant methods and techniques. Any percentage of deaths higher than this number should be a warning of substantial problem somewhere in the study.

### **Inflammation and Fibrin Deposits**

Current carrier coatings may induce inflammatory responses, though typically dose dependent. This response may be acceptable if the reaction is minimal or mild, and does not accelerate, extend or cause substantial vascular injury or stenosis. However, it is key that investigators demonstrate if such early inflammatory reactions meet the above safety criteria for later time points as well.

### **Neointima and Arterial Injury**

Neointima should be thinner and/or of less cross-sectional area in drug-eluting devices for at least some of the measured time points. In longer term studies (greater than 28 days), neointima should be similar to or less than control bare metal stents for test DES formulations. Histopathology showing excessive injury (grade 2 or more) may occur but should be present in less than 20% of sections. These should be quantified nevertheless, and an assessment made by the pathologist as to whether such injury resulted from the drug/polymer associated inflammation or mechanical injury.

Inadvertent severe injury or granuloma formation occurs for unknown reasons in stent evaluation. This is one important reason to include a bare metal stent in each animal. Conclusions of the study may be made without including such granulomatous stents or severely injured sections. The number of such excluded sections should be stated (typically less than 10%).

### **Assessing Vascular Function and Related Phenomena**

Clinical reports suggest that current DES may be associated with aberrant vasomotor function in adjacent conduit arterial segments and collateral vessels in the arterial perfusion distribution. These changes may impact the vascular patho-physiologic milieu, possibly contributing to late DES thrombosis. Accordingly, determination of vasomotor function either in vivo or in vitro,



or both, may be a valuable ancillary tool for differentiating the long-term performance of novel DES and bioabsorbable DES from the predicate devices by providing additional insights into relevant biological phenomena. In particular, defining the nature of endothelium-dependent relaxation response may provide powerful clues as to the impact of DES and absorbable DES on functional endothelialization. Such assessments can be accomplished *in vivo* by angiography (and by use of novel intracoronary measurement tools, to also measure downstream intramyocardial resistance artery function), or *in vitro* after tissue harvest by organ chamber and myograph apparatus. Parallel biochemical and molecular biological analyses to help elucidate mechanisms of abnormal vasomotor function are clearly useful for providing correlative, supportive mechanistic explanatory information.

## Overall Conclusions

This document is an updated guide for preclinical evaluation of modern DES and direct drug-delivery technology. It is a consensus opinion of active investigators in the field of interventional devices. It will continue to be updated, and as experience gained with preclinical models permits better understanding of the important relationships between the models and the clinical results.

## Additional Resources

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**Table 1**

## Comparison of Preclinical Guidelines: USFDA and Expert Consensus Preclinical Consensus

Parameter	USFDA	Expert Consensus Document
Safety and efficacy testing	Safety primary, efficacy secondary endpoints	Safety primary, efficacy secondary end points
Model	Artery without prior injury 1 and 6 months minimum	Artery without prior injury 1 and 6 months, later desirable
Stent overlap	Overlapping and long stents desirable	Overlapping and long stents desirable
Drug toxicity dose and safety margin	Establish dose ranges, safety margin, and establish the lower limit of toxic dose	Dose ranging estimate important. Establish low- and high-dose ranges and safety margin
Drug toxicity IV administration	IV test administration may be beneficial	Complexity of IV administration for 2-phase kinetics and local delivery extrapolation may be of limited utility
Drug toxicity exposure	Establish degree of systemic, local vascular, and myocardial exposure	Establish degree of systemic, local vascular, and myocardial exposure
Device system evaluation	Evaluate stent, stent/polymer, plus drug. Competitive drug-eluting stent is acceptable	Evaluate components separately and together. Control stents should be bare metal and polymer only
Safety and efficacy evaluation	Principal reason for preclinical study is safety assessment	Principal reason for preclinical study is safety assessment. Efficacy may be available also

**Table 2****In Vitro and In Vivo Drug Release Characteristics**

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In vitro half-life estimate, $t_{1/2}$ , where half of all available drug has been released
In vivo peak tissue and blood concentrations and a time-course demonstration of drug remaining in the stent
In vivo $t_{1/2}$ estimate using a minimum of 5 time points each and 3 separate stents
In vivo terminal arterial tissue elimination time
A dose range showing subtherapeutic to maximal practical or toxic levels. A safety margin should be estimated and justified by measured data
Drug concentrations in blood, coronary artery, and myocardium beneath the stent over time points from immediately after implantation until near-complete drug elution. Drug concentration in myocardium supplied by the stented artery, liver, kidney, and lung, measured at necropsy
Optional: in vivo pharmacokinetics
Additional time points to more fully characterize drug release into artery tissue
Drug levels in arterial tissue proximal and distal to stent
Drug levels in myocardial tissue proximal and distal to stent

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**Table 3**

## Histopathologic Injury Score Quantification

Score	Injury
0	Internal elastic lamina intact; endothelium typically denuded, media compressed but not lacerated
1	Internal elastic lamina lacerated; media typically compressed but not lacerated
2	Internal elastic lacerated; media visibly lacerated; external elastic lamina intact but compressed
3	External elastic lamina lacerated; typically large lacerations of media extending through the external elastic lamina; coil wires sometimes residing in adventitia

**Table 4****Important Components for Testing Drug-Eluting Stents**

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Clinical problems (death, fever, infection, rash, inactivity, poor appetite, intolerance) and reasons
Stent thrombosis and/or infarctions
Myocardial infarction, acute and/or healed
Cellularity and description of drug eluting vs control media
Medial area/presence or absence of medial thinning comparison
Inflammation at stent strut sites
Vascular reaction to polymer/carrier
Endothelialization
Fibrin presence/degree
Calcification
Angiographic and histopathologic stenosis

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**Table 5****Important Histopathologic Efficacy Features of Drug-Eluting Stents**

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Continuous variables
Lumen area
Percent area stenosis
Neointimal area
Neointimal thickness
Medial thickness at stent strut sites
Medial thickness between stent struts
Remodeling
Dichotomous variables
Injury
Inflammation

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