

Clinical Trials in Coronary Angiogenesis: Issues, Problems, Consensus An Expert Panel Summary

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Abstract—The rapid development of angiogenic growth factor therapy for patients with advanced ischemic heart disease over the last 5 years offers hope of a new treatment strategy based on generation of new blood supply in the diseased heart. However, as the field of therapeutic coronary angiogenesis is maturing from basic and preclinical investigations to clinical trials, many new and presently unresolved issues are coming into focus. These include in-depth understanding of the biology of angiogenesis, selection of appropriate patient populations for clinical trials, choice of therapeutic end points and means of their assessment, choice of therapeutic strategy (gene versus protein delivery), route of administration, and the side effect profile. The present article presents a summary statement of a panel of experts actively working in the field, convened by the Angiogenesis Foundation and the Angiogenesis Research Center during the 72nd meeting of the American Heart Association to define and achieve a consensus on the challenges facing development of therapeutic angiogenesis for coronary disease. (*Circulation*. 2000;102:e73-e86.)

Key Words: angiogenesis ■ coronary disease ■ growth substances ■ revascularization ■ clinical trials

I. Biology of Therapeutic Angiogenesis

Issues

Three different processes may contribute to the growth of new blood vessels: vasculogenesis, arteriogenesis, and angiogenesis.^{1,2} Vasculogenesis is the primary process responsible for growth of new vasculature during embryonic development³ and may play a yet-undefined role in mature adult tissues.^{4,5} It is characterized by differentiation of pluripotent endothelial cell precursors (hemangioblasts or similar cells) into endothelial cells that go on to form primitive blood vessels. Subsequent recruitment of other vascular cell types completes the process of vessel formation.³ Arteriogenesis refers to the appearance of new arteries possessing fully developed tunica media.⁶ The process may involve maturation of preexisting collaterals or may reflect de novo formation of mature vessels. Examples of arteriogenesis include formation of angiographically visible collaterals in patients with advanced obstructive coronary or peripheral vascular disease. All vascular cell types, including smooth muscle cells and pericytes, are involved. Angiogenesis is the process

responsible for formation of new vessels lacking developed media.¹ Examples of angiogenesis include capillary proliferation in the healing wound or along the border of myocardial infarction. Table 1 summarizes the biology of these 3 processes.

The occurrence of both angiogenesis and arteriogenesis has been demonstrated conclusively in a variety of animal models,^{7,8} as well as in patients with coronary disease.^{9,10} The occurrence of vasculogenesis in mature organisms remains an unsettled issue. As of this writing, it is thought to be unlikely that this process contributes substantially to the new vessel development that occurs spontaneously in response to ischemia or inflammation or as a response to growth factor stimulation.

Tissue ischemia per se may not be the key stimulus in initiation of the angiogenic response. Few patients demonstrate ongoing chronic myocardial ischemia, and most likely the majority of patients with diffuse multivessel disease do not develop tissue-level ischemia in the absence of provocation. Inflammation and shear stress may be much more

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TABLE 1. Three Types of Neovascularization

	Vasculogenesis	Arteriogenesis	Angiogenesis
Cell types involved	Endothelial stem cells	Endothelial cells; smooth muscle cells; pericytes; other	Endothelial cells
Primary stimulus	Development	Not known (Inflammation ?)	Inflammation and ischemia
End result	Fully formed vessels	Arterioles	Capillaries
Occurs in adult tissues	Not clear (minimal?)	Yes	Yes
Contribution to effective perfusion	Not clear (minimal?)	Major	Minor
Growth factors involved	VEGF, Ang-1, Ang-2	PDGF, Ang-1, Ang-2, FGFs (?)	FGF-1, FGF-2, FGF-4, FGF-5, VEGF-1, VEGF-2, VEGF-3

Ang indicates angiopoietin; PDGF, platelet-derived growth factor.

important stimuli,^{11,12} and little angiogenesis takes place in the absence of inflammation. Suppression of inflammatory responses, due to genetic abnormalities, pathophysiological processes, or pharmacotherapy, may adversely affect the ability to induce new vessel growth.¹³

Another critical issue is whether nonischemic myocardium will respond to growth factor stimulation. A considerable body of literature points to nonischemic tissues being largely unresponsive to angiogenic stimuli. This may result not so much from the lack of endogenous growth factors but from alterations in extracellular matrix, the presence of endogenous inhibitors such as angiopoietin II, and the absence of expression of growth factor receptors and other signaling molecules involved in angiogenic signaling.

Problems

- What is the role of each of these processes in neovascularization observed in coronary artery disease?
- What are the stimuli specific to each of these processes?
- Are there growth factors capable of selectively inducing each of these processes?
- What should be the goal of therapeutic angiogenesis?
- What are the beneficial and adverse implications with regard to selection and delivery of angiogenic growth factors?

Consensus

- Arteriogenesis is the preferred type of neovascularization for purposes of restoring myocardial perfusion.
- The role of vasculogenesis in mature tissues has not been established.
- No process-specific stimuli or growth factors have been identified at present. Native collateralization is a complex process that involves multiple levels of stimulators, inhibitors, and modulators. Therefore, for a single growth factor to induce therapeutic angiogenesis, an entire self-propagating cascade of proliferative, migratory, chemotactic, and inflammatory processes must be initiated.
- Combination growth factor therapy or use of master switch genes may be optimal for clinically beneficial therapeutic angiogenesis.

II. Patient Selection

Issues

At present, the majority of patients selected for trials of therapeutic angiogenesis have undergone multiple failed revascularization attempts, including CABG surgery and percutaneous coronary interventions. These individuals may represent failures of natural angiogenic responses and may be particularly resistant to stimulation of neovascularization.¹⁴ Although a variety of considerations have led to selection of patients with advanced coronary artery disease, in practice this may not be the optimal population suitable for proof-of-concept for growth factor therapy. Even if fully successful, therapeutic angiogenesis may not achieve the same magnitude of benefit that can be achieved with optimal mechanical revascularization of a large vessel. Furthermore, the prolonged time course (2 to 3 weeks) of new vessel development after initial therapy likely precludes application of this approach to patients with threatened occlusion of proximal vessels. Therefore, the following patient groups may represent the ideal candidates for induction of therapeutic angiogenesis: those with single, long-standing occlusion(s) of proximal coronary arteries subtending viable myocardium; multivessel diffuse disease with evidence of inducible ischemia and myocardial viability; presence of adequate feeder vessels; and presence of adequate distal runoff.

If successful in these groups, therapeutic angiogenesis may be preferred in patients with coronary anatomy that is less than ideal for angioplasty or stenting and may potentially replace bypass surgery in a significant number of such patients. In addition to these anatomic considerations, a number of presently unknown genetic factors may play a role in the ability to respond to angiogenic stimulation.

Recently, it has also been recognized that a number of common medications may potentially interfere with the angiogenic process,¹⁵ including drugs that are commonly used in cardiac patients, such as spironolactone,¹⁶ captopril,¹⁷ isosorbide dinitrate,¹⁸ lovastatin,¹⁹ bumetanide, and furosemide.²⁰ Even aspirin, a universally used cardiac drug with proven benefit, may have angiogenesis-inhibitory effects.¹³ Some significant noncardiac drugs with antiangiogenic activity include cyclooxygenase-2 inhibitors^{13,21} and the antibiotics clarithromycin²² and minocycline.²³ Other confounding factors may include age,²⁴ hypercholesterolemia,²⁵ smoking,

diabetes, and the presence of endogenous (circulating or local) angiogenesis inhibitors.²⁶

Of equal importance in the design of clinical trials are the proper exclusion criteria. Presently, all patients with prior history of cancer (except for curable skin cancers) or proliferative retinopathies are excluded because of theoretical concerns of inadvertent stimulation of pathological angiogenesis with therapeutic growth factors. Because of concern about renal toxicity, trials with fibroblast growth factor II (FGF-2) have excluded patients with abnormal baseline renal function or significant proteinuria. Additional concerns relate to the state of the coronary plaque at the time of growth factor therapy, because growth factors may potentially stimulate angiogenesis in the plaque that may in turn precipitate plaque rupture, leading to an acute coronary syndrome. Indeed, anecdotal experience with FGF-2 suggests that this scenario may take place. Vascular endothelial growth factor (VEGF) administered shortly after transluminal angioplasty in one animal study resulted in increased neointimal formation. Similar concerns have been expressed with regard to FGFs, although several studies have suggested that FGF-1 and FGF-2 may actually lower the extent of neointimal formation by promoting vessel reendothelialization. It should be underscored that all of these studies have been performed in normocholesterolemic animals. The effect of growth factors on the arterial wall in hypercholesterolemic animals has not been studied adequately.

Problems

- Patients selected for current trials may represent the group that is least likely to respond.
- The nature and role of genetic factors are not accounted for in post hoc analyses of efficacy.
- Concurrent use of medications that may function as angiogenesis inhibitors may occur.
- The necessary extent of cancer screening has not been defined.

Consensus

- Randomization should account for age, sex, and extent of hypercholesterolemia, as well as the magnitude of the native angiogenic response (extent of endogenous collateralization). This can be achieved by performing randomization for the most important confounder and by enrolling sufficiently large numbers of patients (>400 per study arm) such that other unknown factors will be evenly distributed.
- Drugs that may plausibly inhibit angiogenesis (cyclooxygenase-2 inhibitors, furosemide, isosorbide, and captopril) must be accounted for in study design and especially in the sample size calculation, because some of these medications cannot be stopped for the duration of the study.
- Cancer screening should include a comprehensive physical examination, mammography (in women), prostate-specific antigen test (PSA) (in men), sigmoidoscopy in patients of both sexes if aged >50 years, and no negative examination within the prior 5 years.
- Patients with prior malignancies should be excluded.
- Patients with angina at rest should be excluded.

III. Trial End Points

Issues

Trial end points should assess clinical benefit to the patient and also provide a realistic assessment of the mechanism of effect. The choice of end points is heavily influenced by whether therapeutic angiogenesis will prove to be a curative or palliative treatment strategy. If this approach is purely palliative, a substantial improvement in quality of life would be necessary to justify expensive and potentially invasive therapy. On the other hand, documentation of increased survival might justify far greater risks associated with this treatment. Regardless of the extent of efficacy, a clear-cut mechanism of action should be demonstrated for a proposed treatment to gain acceptance in the medical community. At a minimum, this must include demonstration of improved myocardial perfusion and possibly improvement in global and/or regional left ventricular function or increased collateral vessels on coronary angiography. The utility of perfusion imaging and coronary angiography is discussed in separate sections of this document, whereas this section concentrates on the remaining end-point measures.

Exercise Tolerance Testing

Exercise tolerance testing has been used as the primary end point in a number of phase 1 and phase 2 trials. One important limitation of this end point is the high variability in exercise performance on a day-to-day basis among patients with coronary artery disease. Exercise treadmill time may also be influenced by many factors beyond angina, such as claudication from concomitant peripheral vascular disease, pulmonary disease, deconditioning, and motivation. Moreover, although exercise time has often been used in studies of antianginal therapies in stable angina trials, those studies generally included patients with mild stable angina and normal left ventricular function. In contrast, the populations participating in current therapeutic angiogenesis trials are predominantly patients with long-standing chronic coronary artery disease, most of whom have had prior bypass surgery and/or moderate degrees of left ventricular dysfunction, which may also importantly affect exercise time.

The limitations of maximal treadmill time as a trial end point are underscored by clinical studies of therapeutics for heart failure. Agents with consistently proven favorable effects on long-term outcomes, such as ACE inhibitors and β -adrenergic blockers, have not shown consistent positive effects on maximal treadmill time, whereas other therapies with an adverse effect on survival (such as milrinone) may improve exercise time.²⁷ Thus, although exercise duration is often used as the primary efficacy end point in angiogenesis trials, it is unclear whether this will prove to be a robust measure that reflects clinical improvement resulting from therapeutic angiogenesis.

Finally, another substantial practical limitation of exercise testing is the fairly subjective indications for termination of exercise (no matter how rigorously specified). Therefore, blinding of investigators and patients is mandatory, as is the exclusion of patients who demonstrate high variability (>30%) in exercise duration on 2 consecutive tests.

Prolonged Survival

Prolonged survival is one of the unequivocal goals of treatment for patients with chronic ischemic heart disease. Although there is general agreement that reduction of long-term mortality would be a desirable goal of coronary angiogenesis, the use of survival as a primary study end point that requires a prolonged study period is relatively impractical in the early stages of clinical research, in which the main goals are generally to identify the appropriate dose of therapy and the optimal delivery mode. Nonetheless, patients with extensive coronary artery disease who are not candidates for conventional revascularization techniques have substantial excess mortality. Consequently, reduced long-term mortality may be a reasonable goal for large-scale phase 3 or phase 4 clinical trials once the mechanism and appropriate dosing of a therapeutic agent have been established.

Improvement in Health-Related Quality of Life

Improvement in health-related quality of life (HRQOL) is an important therapeutic objective for patients with chronic ischemic heart disease, and it may be well suited for use as an end point in clinical research. There is little question that improvement in health status or quality of life is an important therapeutic objective of coronary angiogenesis for both patients and clinicians. In fact, most patients who seek alternative treatments for chronic ischemic heart disease identify improved quality of life as their most immediate goal. An important advantage of quality of life/health status as an end point for clinical trials in coronary angiogenesis is that improvements in health status tend to be realized in the relatively short time frames that are required for phase 2 and 3 studies. Although the research community (and the Food and Drug Administration) have traditionally relied on exercise tolerance and related measures of inducible myocardial ischemia (eg, perfusion imaging) as end points for coronary angiogenesis trials, such measures are poor surrogates for improved quality of life from the patient's perspective.²⁸ Despite the clear advantages to HRQOL as an end point for coronary angiogenesis research, there are important challenges to its widespread use and acceptance. Foremost among these is the absence of a single definition of quality of life that is meaningful and applicable to all patients and disease states. To overcome this limitation, a multidimensional approach using a combination of disease-specific and generic quality-of-life measures has been proposed.^{29,30}

Disease-specific measures such as the Canadian Cardiovascular Society (CCS) anginal classification or the Seattle Angina Questionnaire (SAQ) focus on those domains of health that are most relevant to the disease under investigation. For patients with chronic ischemic heart disease, these domains would include functional limitations and symptoms such as angina and dyspnea. The principal advantages of disease-specific quality-of-life measures is that these outcomes tend to be most relevant to both patients and clinicians and may thus be easier to interpret. In addition, disease-specific measures tend to be more responsive to modest changes in health than generic measures³¹ and thus can be an efficient study end point.

Generic health status measures such as the Medical Outcomes Study SF-36 or SF-12 or the Sickness Impact Profile are designed to summarize a spectrum of concepts of health and quality-of-life issues so as to be broadly applicable across a wide variety of disease states and patient populations. Such measures have the advantage of capturing a more comprehensive assessment of the health effects of a particular disease or intervention than would be detected by either disease-specific measures or traditional "hard" clinical end points. For example, studies have demonstrated that bypass surgery has an effect on a broad range of health status dimensions, including physical activity, role function (ability to perform one's usual activities), psychological functioning, anxiety, vitality, and sense of well-being.³²⁻³⁴ Although there are correlations between traditional physiological parameters such as exercise test duration and global health status indicators, these correlations are modest at best.³⁴ Thus, it is critical to measure health status directly when one is assessing a treatment whose major impact is on quality of life. Moreover, the growing experience with generic health status measures provides the framework and database necessary for quantifying the impact of a new therapy on health relative to other accepted medical interventions.

Preference-based assessment is another measure that is gaining increasing importance in the evaluation of new medical technologies. These measures attempt to assign each patient's health state a single value ("utility") that reflects the individual's preference for his or her current state of health relative to perfect health and are designed to explicitly account for the types of risk-benefit tradeoffs inherent in all medical decision making. Such measures are particularly important for evaluation of the cost-effectiveness of new medical technologies, which is becoming an increasingly important determinant of patterns of use and insurance coverage. Although assessment of health state utilities has traditionally required complex, labor-intensive interviews, recently several multi-item questionnaires, including the Health Utilities Index³⁵ and the EuroQOL,³⁶ have been developed and validated for these purposes.

Despite its obvious importance as a meaningful clinical end point for coronary angiogenesis, HRQOL may be viewed with skepticism as a valid scientific end point for biomedical research. The principal reason for such skepticism relates to the perception of quality of life as a "soft" outcome. Although this criticism can certainly be raised for early scales such as the New York Heart Association or CCS classification schemes,³⁷ contemporary instruments such as the SAQ^{31,38} and the SF-36 Health Status Questionnaire³⁹ are highly reliable and reproducible, do not require human input for scoring, and have been validated against external standards by standard psychometric techniques. For example, the physical limitations domain of the SAQ has been shown to correlate closely with treadmill exercise duration, whereas the anginal frequency domain has been validated against nitroglycerin pill counts.³⁸ Moreover, preliminary research suggests that several domains of the SAQ are independent predictors of long-term survival in patients with chronic ischemic heart disease (J.A. Spertus, MD, oral personal communication, 2000). The only substantial impediment to

the use of quality of life as an objective outcome in clinical research is the placebo effect. However, use of a double-blind, placebo-controlled design in angiogenic trials should control for this effect. Thus, to the extent that the constructs they measure are adequate reflections of specific health status domains, and so long as a valid placebo is used in the trial design, the available quality-of-life instruments fulfill all of the traditional criteria of an “objective” outcome measure.

The major remaining limitation of health status/quality of life as an outcome measure for clinical research relates to challenges in interpretation of between-group differences in these measures. Although clinicians have a “feel” for the meaning of a 2-class improvement in CCS anginal grade or a 1-minute improvement in treadmill exercise time, the precise meaning of a 10-point improvement on the SAQ anginal frequency scale or a 4-point difference in the SF-36 physical function scale is more problematic. These limitations primarily reflect a lack of familiarity with the newer health status instruments, however, rather than an intrinsic failing of the instruments per se. As such, their interpretation may be facilitated by comparison of the benefits of a new intervention such as coronary angiogenesis with the benefits of an established treatment such as PTCA using the same instruments (eg, reference-based interpretation). Alternatively, interpretation of continuous health status measures may be enhanced by categorizing changes in the specific domains on the basis of established performance criteria. For example, previous studies have demonstrated that individual improvements of 8 to 10 points on any of the SAQ subscales or >4 points on the SF-36 physical component scale are “clinically meaningful” to patients. Preliminary research suggests that each 1-class improvement in CCS anginal scale is approximately equivalent to a 12-point improvement on the SAQ anginal frequency scale (D.J. Cohen, MD, MSc, et al, unpublished data, 2000). By providing a categorical description of each patient as “improved,” “unchanged,” or “worse,” such threshold-based analyses can enhance the clinical interpretability of inherently continuous outcome measures.

An additional caveat in the application of HRQOL to therapeutic angiogenesis trials is that most of the quality-of-life scales have been designed and implemented in revascularization or medical therapy trials in patients who are normally distributed along the severity of ischemic heart disease scale. Patients enrolled in angiogenesis and laser myocardial revascularization trials may start much lower on that scale, because most have angina refractory to medical therapy and not amenable to standard revascularization. The improvement they may experience may not be detectable by current HRQOL questionnaires but may be clinically important to them for their daily living. Therefore, the range of many of the available quality-of-life assessment tools may have to be shifted and designed specifically for this target population.

Problems

- Mortality differences may take several years to become apparent and are unsuited for phase 2 and “proof-of-concept” phase 3 trials.

- There is high day-to-day variability of exercise tolerance testing.
- The indications for termination of exercise testing are subjective by nature.
- No single definition of quality of life is suitable to all patients and disease states.
- Health status and quality of life are generally perceived as “soft” outcomes that are unsuited for quantitative, biomedical research.
- Quality-of-life outcomes are highly subject to manipulation by the placebo effect.
- Interpretation of observed differences in newer quality-of-life measures is difficult for both clinicians and patients.

Consensus

- Mortality should be tracked for safety purposes in early clinical trials and should be considered as an important end point for evaluation of therapeutic angiogenesis once an appropriate agent and dosing regimen have been established through phase 2 and phase 3 trials.
- Exercise stress testing should be performed in a double-blinded manner.
- Patients with variable (>30% difference) baseline test results should be excluded from clinical trials.
- Although 1 or at most 2 quality-of-life measures should be prespecified for analytic purposes, multiple domains of health should be assessed, including disease-specific and generic instruments. If an economic evaluation of the technology is also planned, inclusion of a preference-based quality-of-life (ie, utility) measure is also desirable.
- Use of reliable, validated instruments that can be self-administered and scored by standard algorithms is essential to provide an objective assessment of health status and quality of life.
- Because of the strong influence of the placebo effect, blinding of the patient, the referring physician, and the investigator to the specific treatment is critical to acceptance of quality-of-life measures as a valid study outcome.
- Development of standards for “clinically meaningful” or “clinically substantial” changes is necessary to facilitate interpretation of trial results.
- An angiogenesis-specific quality-of-life assessment tool should be developed.

IV. Noninvasive Myocardial Perfusion Imaging

Issues

Noninvasive imaging methods have been used in many of the early clinical trials of therapeutic myocardial angiogenesis to explore mechanisms of symptomatic benefit and to provide evidence that such therapy does indeed enhance blood flow to ischemic myocardium. Thus, imaging of myocardial perfusion, left ventricular function, or both has been incorporated into the study design of many of the phase 1 trials and ongoing phase 2 trials. Unfortunately, only limited data are available at the present time that have been obtained primarily from small uncontrolled trials or registries, and hence no definitive conclusions can be reached regarding the reliability

of these methods as study end points or their future clinical efficacy in the serial evaluation of patients who are undergoing angiogenesis therapy.

SPECT Myocardial Perfusion Imaging

Myocardial perfusion imaging with ^{201}Tl or $^{99\text{m}}\text{Tc}$ sestamibi is firmly established for the diagnosis of ischemic heart disease and for detection of improved blood flow after revascularization of epicardial coronary arteries. Hence, improved myocardial perfusion at rest or with exercise or pharmacological stress is an anticipated finding in patients who have undergone coronary bypass surgery or percutaneous coronary intervention. However, the usefulness of perfusion imaging in the detection of improved perfusion related to enhanced collateral supply remains to be established. There are a number of conceptual concerns regarding the use of single photon emission computed tomography (SPECT) to assess improved blood flow resulting from angiogenic therapy. First, patients who are candidates for angiogenesis trials thus far have been patients with advanced multivessel disease, often with impaired left ventricular function, and many such patients are unable to attain a maximal heart rate with exercise, thereby limiting a full appreciation of the extent of ischemic myocardium on baseline studies. In addition, for purposes of interpretation of serial SPECT studies, it is virtually impossible to match levels of myocardial demand on repeat exercise tests, because heart rate and blood pressure responses are difficult to duplicate. Thus, in the design of prospective angiogenesis trials, it may be preferable to use pharmacological vasodilator stress with dipyridamole or adenosine, which will optimize identification of the extent of ischemic myocardium and provide a more reproducible means to study myocardial flow reserve in serial studies. It is also conceivable that therapies that are effective in increasing collateral blood flow delivery may be difficult to fully detect by either pharmacological or exercise stress testing, because collateral-dependent myocardium has limited flow reserve^{40–42} and the potential to create a myocardial steal.⁴³ This suggests that increased collateral supply may be reflected more accurately and more reproducibly by changes in resting perfusion than by changes in perfusion during exercise, vasodilator stress, or administration of dobutamine.

There are other conceptual issues regarding the use of SPECT in angiogenesis trials beyond the form of stress testing. The sensitivity and spatial resolution of SPECT for detecting subtle improvement in perfusion are a cause for concern. SPECT is highly sensitive for detecting and localizing large areas of ischemia or infarction in the distribution of the large coronary arteries, and changes in both the magnitude of ischemic myocardium and the severity of ischemia after revascularization have been well studied in large populations. However, this may not be applicable in patients in whom small increases in blood flow through collaterals, primarily to the subendocardial layer, have been stimulated by angiogenic mechanisms. The spatial resolution of SPECT cannot differentiate transmural gradations in blood flow and flow reserve and thus cannot differentiate major changes in flow in the subendocardium layer alone from

relatively minor changes in flow that are uniform throughout the entire thickness of the myocardial wall.

The above concerns are especially pertinent with regard to the use of automated computer programs that have been developed to identify and quantify extent and severity of ischemic myocardium. Although these algorithms appear to accurately detect which patients have coronary artery disease, and they certainly provide complete objectivity, their ability to detect very subtle serial changes in perfusion may be suboptimal. The trained human eye may be more adept at integrating small serial changes in severity and extent of ischemia. It is noteworthy that 2 trials of transmural laser revascularization that used thallium SPECT imaging to assess improvement in perfusion reported disparate results. The trial that used an automatic computer analysis of the thallium data reported no effect of therapy on the number of ischemic defects,⁴⁴ whereas the trial designed around the blinded interpretations of a skilled nuclear cardiologist reported a significant improvement in ischemic defects in patients treated with laser revascularization compared with those treated with medical therapy.⁴⁵ Despite these conceptual concerns, the available early evidence from phase 1 trials indicates that SPECT imaging may be able to detect improvement in myocardial blood flow in patients undergoing angiogenic gene or protein therapy.^{46–49}

Positron Emission Tomography

Although a few patients have undergone imaging with positron emission tomography (PET) before and after angiogenic therapy, there are currently no clinical trial data with this imaging technique. PET has several advantages over SPECT that would be beneficial in clinical trials of new therapies to stimulate collateral growth. Unlike SPECT, attenuation correction is a routine process with PET imaging that significantly improves image quality, and PET also has the potential to detect more subtle changes in blood flow and flow reserve than might be anticipated with SPECT. Such subtle but important changes in flow reserve have been demonstrated in 2 PET studies demonstrating improved coronary flow reserve with lipid-lowering therapy,^{50,51} presumably related to improved endothelial function. PET, like SPECT, suffers from limited spatial resolution, with the inability to differentiate subendocardial versus transmural flow changes (and hence the potential for minor flow changes limited to the endocardial layers to go undetected), but PET is the only method with which to measure absolute blood flow. The limited availability and expense of PET prevent its uniform application in large-scale clinical trials, but it is anticipated that PET will be used in substudies of angiogenesis trials in the near future.

Magnetic Resonance Imaging

MRI has enormous potential to assess myocardial structure, function, and blood flow. Myocardial blood flow assessment with gadolinium-based contrast agents is slowly emerging as a clinical reality, although this field is clearly less established than the nuclear cardiology–based perfusion methods. As the MRI evaluation of myocardial perfusion evolves, clinical trial end points based on MRI assessment of blood flow, flow reserve, and collateral blood flow will gain greater accep-

tance. There are currently no MRI-based perfusion agents that are retained by the myocardium, and so MRI assessment of perfusion is based on first-pass assessment of contrast appearance and washout rates. The advantage of MRI is the exceptional spatial resolution, which allows assessment of the transmural flow gradient and the ability to assess changes in subendocardial perfusion and perfusion reserve. Animal models of coronary stenosis have validated MRI measurement of late contrast appearance as a measure of collateral-dependent myocardium, which has been reduced with VEGF administration.⁵²⁻⁵⁴ Phase 1 randomized, placebo-controlled trial results with gadolinium-DTPA in patients treated with local perivascular administration of recombinant FGF-2 at the time of bypass surgery have demonstrated the potential importance of MRI perfusion imaging by reporting significant improvement in delayed contrast arrival in patients treated with recombinant FGF-2.⁵⁵ MRI substudies in phase 2 trials are in progress and will be reported in the near future.

Problems

- SPECT scans have poor spatial resolution, with inability to image the subendocardium selectively.
- SPECT perfusion imaging has a “relative” nature.
- The results of quantitative versus semiquantitative SPECT assessment are discordant.
- A lack of standards exists with regard to MR and PET perfusion imaging (acquisition and analysis).
- There is a paucity of clinical experience with PET and MR perfusion imaging.

Consensus

- Perfusion imaging is necessary for demonstration of angiogenic “efficacy.”
- MR perfusion imaging may eventually be the best means of perfusion assessment.
- Pharmacological stress testing is preferred to exercise protocols.
- Semiquantitative SPECT analysis appears to be the most available and accepted tool at present, despite its many limitations, and should only be performed in a blinded fashion in double-blind placebo-controlled trials.

V. Coronary Angiography

Issues

The advantages of coronary angiography in trials of therapeutic angiogenesis relate to its ability to (1) assist in stratification of disease severity, (2) assist in patient selection and randomization, (3) identify the extent of disease progression (an adverse effect of treatment), and (4) document the appearance of new vessels. Considerable challenges are encountered with each of these applications. Patient stratification and randomization are confounded by the imprecise knowledge of the natural history of disease in individual patients and the inability to predict rate and extent of angiographic disease progression. The ability to measure disease progression is compromised by the diffuse nature of atherosclerosis and the inability to fully survey the coronary

tree quantitatively. The angiographic appearance of collateral vessels as an end point is limited by the variability of hand injections of contrast, spatial resolution of the technique, and typically, extensive preexisting collateral networks.⁹ Digital subtraction angiography and quantification of myocardial capillary “blush” have not been established as reliable imaging modalities.

Problems

- Angiography has limited spatial resolution.
- Assessment of collateral vessels is subjective.

Consensus

- Angiography is an essential tool for trial eligibility screening.
- Angiography may be useful in identifying treatment complications.
- Angiography may be useful in identifying new collateral growth.

VI. Delivery

Issues

Translation of growth factor therapy, which has been very effective in animal studies, to clinical trials requires a practical delivery strategy. This requirement essentially eliminates all forms of prolonged or frequent repetitive intracoronary infusions. Local perivascular delivery is easily adaptable to clinical trials but requires open-chest surgery, although it could potentially be accomplished thoracoscopically.⁵⁶ One such form of delivery is implantation of heparin alginate capsules that provide prolonged (4 to 5 weeks) first-order kinetics release of the growth factor from the polymer.^{55,57-59} The capsules are easily implantable and do not provoke an inflammatory response.⁶⁰⁻⁶² A potential advantage of perivascular delivery is the absence of both the endothelial barrier and the rapid washout typical with intravascular administration.⁵⁹

An alternative approach to perivascular administration of basic fibroblast growth factor (bFGF) involves intrapericardial instillation of the growth factor. A major advantage of this approach is that it can be accomplished via a catheter, obviating the need for open-chest surgery.^{63,64} However, current clinical application of intrapericardial delivery is limited to a small number of patients now enrolled in coronary angiogenesis trials because of the high prevalence (80% to 90%) of prior CABG surgery in this group of patients.

The feasibility of short-duration intracoronary or intravenous infusions and endomyocardial injections has also been tested in animal models. Intravenous infusions are appealing because of their practicality, low cost, and applicability to broad groups of patients. Furthermore, treatment can be repeated easily and may not require any special facilities. The downside includes systemic exposure to a growth factor and potential for side effects such as nitric oxide-mediated hypotension.^{65,66} Intracoronary infusions are easily performed in any cardiac catheterization laboratory and are also appli-

TABLE 2. Gene vs Protein Therapy

Gene Therapy	Protein Therapy
<p>Pro</p> <ul style="list-style-type: none"> ● Sustained production of angiogenic factor resulting in prolonged exposure to elevated levels. ● Capacity for local delivery, thus local therapy; less systemic exposure to angiogenic factor. ● Can be accomplished with a single administration. ● Angiogenic factor production and secretion can be directed to specific cell type (eg, cardiac myocyte). <p>Con</p> <ul style="list-style-type: none"> ● Introduction of foreign genetic material. ● Exposure to viral vectors with concomitant risk of inflammatory response, viral persistence, and in vivo recombination. ● Potential for non-target-cell gene delivery. ● Inability to precisely modulate gene expression and thus angiogenic factor levels with present vector systems. ● Potential for inactivation and/or inflammatory response at readministration. ● Potential for long-term, low-level systemic exposure to secreted angiogenic factors. 	<p>Pro</p> <ul style="list-style-type: none"> ● Finite temporal exposure to angiogenic factor. ● Titratable dosing of angiogenic factor exposure. ● No exposure to exogenous genetic material. ● No exposure to viral vectors. ● Readministration may be easier; decreased risk of inflammatory response or immune inactivation at the time of repeat dosing. ● Modulation of protein structure and/or combination with slow-release delivery systems may abrogate issue of protein stability. <p>Con</p> <ul style="list-style-type: none"> ● Short serum half-life; finite tissue half-life; consequently shorter exposure periods. ● May require repeated administration. ● Higher short-term systemic exposure when delivered intravascularly.

cable in most patients with coronary artery disease. However, the need for left heart catheterization limits this approach to a single session or, at most, infrequent repetitions. Although somewhat more “local” than intravenous infusions, intracoronary infusions are also likely to result in systemic exposure to the growth factor and may precipitate systemic hypotension.^{65,67} A variation on the same theme is transvascular intracoronary administration with a local delivery catheter.⁵⁴ This approach, although potentially feasible, remains experimental at this time and is still associated with significant systemic recirculation.

Detailed evaluation of tracer-labeled growth factor uptake and retention in the myocardium and its systemic distribution after intracoronary and intravenous infusions has demonstrated that both forms of delivery were associated with relatively low uptake in the target (ischemic) area of the heart. Thus, 1 hour after injection, 0.9% of the injected bFGF was found in the ischemic myocardium after intracoronary and 0.26% after intravenous administration. Perhaps more importantly, only very small amounts of the growth factor remained in the myocardium 24 hours later (0.05% for intracoronary and 0.04% for intravenous administration).⁶⁸

Intramyocardial delivery of growth factors is the least-evaluated form of therapy at this time. The appeal of this mode of delivery includes the possibility of targeting the desired areas of the heart, likely higher efficiency of delivery, and prolonged tissue retention. The drawbacks are its invasive nature, a requirement for highly specialized equipment, and the need for a high skill level of the operator. Furthermore, no conclusive data regarding the physiological efficacy of this mode of administration are available to date. On a positive note, tracer-labeled growth factor uptake and retention are much better with intramyocardial than with intracoronary or intravenous delivery.⁶⁹

Problems

- Multiple delivery modes (systemic, intravascular, perivascular, or intramyocardial) remain unproven in terms of clinical efficacy and superiority.
- The optimal dose schedule (single versus repeated or sustained delivery) remains unknown.
- The pros and cons of surgical versus percutaneous delivery have not been established.
- No clear means of maximizing tissue and myocardial distribution and retention exists.

Consensus

- Preclinical and clinical studies should be preceded by tissue distribution studies to define the myocardial uptake and retention or expression of growth factor.
- Intravenous delivery is not likely to produce functionally significant angiogenesis.
- Intracoronary delivery may be effective when adequate doses are used.
- Intrapericardial delivery does work but necessitates a normal pericardium (eg, it cannot be used in post-cardiac surgery patients).
- Intramyocardial delivery may provide better myocardial distribution and retention than intracoronary delivery, but its efficacy with regard to either protein or gene administration must be demonstrated.
- Gene therapy may be enhanced with the development of second-generation vectors (eg, regulatable expression, lack of inflammation, and tissue specificity).

VII. Protein Versus Gene Therapy

Issues

Theoretically, angiogenesis can be achieved either by the use of growth factor proteins or by the introduction of genes

encoding these proteins. The argument in favor of a gene therapy approach to stimulate therapeutic angiogenesis holds that gene therapy can overcome the inherent instability of angiogenic proteins by facilitating sustained, local production of these angiogenic factors (Table 2).^{70,71} The arguments against protein therapy follow similar reasoning. There are, of course, related arguments favoring protein therapy over the gene therapy approach.

Sustained Expression

Compared with protein administration by intravascular route, gene transfer can result in longer-term exposure to an angiogenic factor. It is not known, however, whether this is clearly advantageous for a biologic effect. Animal studies suggest that protein therapy can be effective with single administration.^{54,72} One explanation for a biologic effect after single-dose protein exposure is that a cascade of molecular and cellular events constituting an “angiogenic program” is set into motion in susceptible (eg, ischemic) tissues after relatively short-term exposure to an angiogenic protein.⁷³ Another explanation holds that despite a short serum half-life, the tissue half-life of these proteins may be significantly longer. Along these lines, some angiogenic factors, such as VEGF, have potent vascular permeability effects that may facilitate their egress from the microvasculature and consequent tissue deposition after local intravascular (eg, intracoronary) delivery. This may extend their effective half-life and thus obviate the theoretical gene-delivery advantage. Furthermore, by a variety of methods (eg, heparin-alginate beads, slow-release preparations, direct intramyocardial injection, and genetic modification), the effective tissue half-life of angiogenic proteins can be extended.^{55,63} Currently, however, gene therapy appears to hold an advantage over protein administration for sustained exposure to an angiogenic factor.

A key issue is that prolonged exposure to angiogenic stimulation may have considerable safety implications given the critical role of angiogenesis in malignancies and other pathologies. Therefore, the theoretical advantage of gene therapy approaches with respect to longer-term angiogenic-factor exposure hinges on local expression and lack of systemic effects and may embody concomitant safety concerns. Gene-delivery approaches differ in the duration of transgene expression achieved. Plasmid DNA and early-generation adenoviral vectors mediate a rather short-duration expression, whereas other viral vectors (eg, retroviral, lentiviral, and adeno-associated viral [AAV] vectors) can result in very long duration of expression.

The limited duration of transgene expression (≈ 1 to 2 weeks) achieved in the heart with first-generation adenovirus vectors makes them in some manner ideal for angiogenic gene delivery.^{71,74} However, this limited duration of expression is attributed at least in part to an immune response against adenoviral proteins.⁷⁵ Thus, there are concerns about inflammatory responses to these vectors, although this remains controversial, and inflammatory responses may be more likely in some tissues than in others.^{76,77} The issue of immune and inflammatory responses to viral vectors may be overcome by the use of alternative viral vectors (eg, AAV) or newer-generation adenovirus vectors. However, these vectors

may lead to longer-term transgene expression with the concomitant safety concerns associated with prolonged angiogenic stimulation, thus the “gene therapy paradox” in which “safer” vectors result in potentially deleterious prolongation of therapeutic gene expression. To address this issue, vector systems capable of regulated therapeutic gene expression are currently under development.

Local Delivery

Although genes can be delivered locally (eg, direct injection into the myocardium), angiogenic factors such as VEGF or FGF-4 are secreted proteins. Therefore, local gene-delivery and protein production does not limit the secreted protein product to the target tissue. There is likely to be prolonged “leakage” of the angiogenic factor into the systemic circulation. Whether the levels of circulating protein produced in this manner are a safety concern remains unclear, but this phenomenon must be considered when the validity of the local-delivery argument for gene therapy is being assessed. Conversely, there is higher short-term systemic exposure when an angiogenic protein is delivered intravascularly. It remains unknown whether higher-level short-term systemic exposure with intravascular protein delivery or lower-level prolonged exposure potentially related to gene therapy is the more significant safety concern. Further complicating this dichotomy is that systemic exposure after protein delivery may be abrogated by intramyocardial protein injection or other local protein-delivery methods. A final consideration in this category is that viral vector administration, especially by intravascular delivery, can lead to systemic exposure to the vector.⁷⁸ Although gene expression can be directed transcriptionally to a specific cell type (eg, the cardiac myocyte) by use of tissue-specific promoters, this only addresses the issue of gene expression, not delivery. Although only a theoretical consideration at this point, non-target-tissue exposure to viral vectors may carry safety concerns.

Single Administration Versus Recurrent Treatment

Whereas gene therapy approaches are generally thought to be single-administration approaches, there are caveats to this “advantage” as well. First, as discussed, it is currently unclear whether repeated administration of protein will be necessary, especially in light of the various approaches for increasing the tissue half-life of angiogenic proteins. Second, it remains unclear whether “successful” angiogenic therapy by any approach will be stable or will require maintenance or repeated treatments. In the VEGF and FGF-2 protein-delivery clinical trials, there has been no evidence of anti-growth-factor antibody production, and hence it appears that these proteins can be readministered effectively (Reference 65 and M. Pike, MD, oral personal communication, 2000). Whether or not gene delivery, specifically by viral vector administration, can be repeated effectively in the same patient remains unclear. There is significant concern about the effects of neutralizing antibody on viral vector readministration, and there are animal data suggesting that readministration of adenovirus vectors can lead to significant inflammation at the site of initial exposure.⁷⁹ However, this has not been demonstrated in heart after intracoronary or intramyocardial deliv-

ery and may not be an issue with plasmid DNA delivery approaches.

Dosage and Pharmacokinetics

The use of proteins allows administration of precise amounts of growth factors with a well-defined half-life, pharmacokinetics, and safety record. Gene therapy in its present form is associated with much more variability in the levels of the proteins produced and duration of expression.

Safety Concerns

Currently, limited clinical data from protein- and gene-delivery trials suggest that both approaches are safe.^{46,47,55,65,80} However, a great deal more clinical experience will be necessary to address the theoretical safety issues more substantively. Safety concerns about therapeutic angiogenesis center on 2 issues: potentiation of pathological angiogenesis (eg, malignancies) and “bystander” effects of the delivered factor (eg, effects on the kidney or on the atheroma). Gene therapy approaches to therapeutic angiogenesis have additional concerns regarding the introduction of foreign genetic material and exposure to viral vectors. Adenovirus vectors have been associated with inflammatory responses, and recent data suggest that these vectors can persist under certain circumstances.⁸¹ One study⁸² also suggests that earlier-generation adenovirus vectors that retain portions of the E4 adenoviral gene can cause dysregulation of a number of host cell genes. The significance of this finding remains unknown, and these vectors remain in current clinical use. Recently, a patient died after administration of a high dose of recombinant adenovirus vector to the liver.⁸³ Adenovirus has been used safely in other clinical trials, however, and whether this event was related specifically to the vector or was due to another cause is not currently clear. Nonetheless, this experience reestablishes that safety issues are an ongoing consideration. The field of gene therapy is continuously evolving, and newer gene-delivery systems (eg, regulatable nonimmunogenic vectors) will likely be progressively safer. Given that both protein- and gene-delivery approaches have been relatively well tolerated thus far in clinical trials, current safety concerns remain theoretical, and an advantage cannot be definitively attributed to either approach.

Finally, the current paradigm holds that endogenous control of angiogenesis involves an equilibrium between angiogenic stimulation and inhibition. Thus, high local concentrations of an angiogenic factor will tip the equilibrium in favor of neovascularization. This model has been the basis for current clinical attempts to stimulate therapeutic angiogenesis and has been featured in arguments favoring prolonged local exposure to high levels of an angiogenic factor. However, this model is probably substantially oversimplified, and scientific discovery in the area of angiogenesis is proceeding at a rapid pace. As knowledge in this area expands, the issue of gene delivery versus protein delivery may become less important.

Problems: Gene Therapy

- Regulatable gene therapy vectors are not available.
- Gene therapy has a poorly and incompletely understood side effect profile.

- The length and level of gene expression are unpredictable.
- Gene therapy may require complicated delivery modalities for transendocardial approaches.

Consensus: Gene Therapy

- The gene-delivery approach is the only option for certain genes such as transcription factors (eg, HIF-1 α).
- Regulatable vectors with short (5 to 7 days) duration of expression are highly desirable.

Problems: Protein Therapy

- Limited tissue half-life may require sustained-release preparation.
- Protein therapy may require complicated delivery modalities.

Consensus: Protein Therapy

- Protein therapy is closer to practical use than gene therapy.

VIII. Issues Specific to Surgical Trials

Issues

The published angiogenesis trials reported to date involving a surgical delivery approach consist of 4 studies: plasmid-mediated delivery of VEGF 165,⁴⁶ adenovirus-mediated delivery of VEGF 121,⁴⁷ protein-based delivery of FGF-1,⁸⁰ and protein-based delivery of FGF-2 in a sustained-release heparin alginate formulation.⁵⁵ By definition, all of these trials have involved the direct intramyocardial delivery of an angiogenic mediator, with the advantages and disadvantages of this delivery technique compared with intravascular administration (see section VI). Furthermore, all of these trials have adopted the strategy of delivering growth factor to areas of reversible ischemia not amenable to conventional therapies such as angioplasty or bypass. Potentially more sophisticated efforts to specifically treat ischemic “border zones” or to provide pathways for vessel growth have not yet been examined.

The surgical studies reported thus far are limited by several features that might be anticipated in such small phase 1 studies. First, all involve only small numbers of patients. Second, neither of the gene therapy–based trials^{46,47} included a negative control group, and thus a placebo effect and other observer biases cannot be excluded. Finally, 3 of the trials^{47,55,80} are partially confounded by the performance of concomitant CABG surgery as part of the protocol design in at least some of the study patients. Whether safety and efficacy outcomes were related to the angiogenic therapy or to the effects of CABG surgery, in one of the studies⁵⁵ significant enhancement in perfusion was noted in the nonbypassed myocardium compared with negative controls. In addition, positive outcomes have been reported in terms of angina class and antianginal medications, exercise treadmill duration, angiographic scores, and myocardial perfusion assessed by MRI or SPECT scans. Furthermore, long-term data in

some of these studies now extend to the 6- to 12-month interval, beyond which placebo effects are unlikely to be relevant.

These positive preliminary results notwithstanding, the surgical trials raise a number of difficult issues. In particular, growth factor proteins or genes encoding these substances can be administered to patients in conjunction with coronary artery bypass surgery or as sole therapy. The limited experience with sole-therapy trials to date^{46,47} suggests that such an approach is feasible. However, its safety has not been established, and there may be considerable reluctance on the part of referring physicians to enroll patients in such studies or on the part of cardiac surgeons to perform such procedures. It is also extremely unlikely that a randomized trial that would require a control group to receive implantation or administration of a placebo would ever be performed. On the positive side, sole-therapy trials offer the cleanest means for demonstrating therapeutic efficacy. However, given considerations already discussed, this approach may best suited to phase 1 trials.

On the other hand, “CABG Plus”^{47,55,80} studies, although much easier to perform, have a variety of challenges of their own. First, the definition of incomplete revascularization is uncertain. One might define incomplete revascularization as either a failure to graft all diseased arteries >1 mm in diameter or as a failure to graft 1 vessel in each of the 3 major territories. Alternatively, an incomplete revascularization might be defined as an inability to graft an artery perfusing $\geq 25\%$ of the total myocardial surface area. This needs to be standardized, but either, or preferably both, of the latter 2 conditions should be met. A second important preoperative issue is the need to demonstrate viability in the area of incomplete revascularization. Additional technical considerations, including the surgical approach (sternotomy versus lateral thoracotomy), distribution of delivery sites, and the means and timing of administration (before or after anastomoses are completed, on or off bypass, and type of cardioplegia) need to be standardized. Intramyocardial delivery may require echocardiographic guidance to document myocardial injection, especially if done on the beating heart.

Evaluation of efficacy data are an additional challenge in “CABG Plus” trials. Part of the uncertainty arises from the lack of detailed knowledge of clinical history and outcomes among patients with incomplete revascularization⁸⁴ and another part from the imprecision of noninvasive imaging tools. Nuclear perfusion imaging, dobutamine stress echocardiography, digital subtraction angiography, and MR imaging have been used to assess the changes in perfusion in these studies.^{47,55,80} All have their limitations as well as advantages. In particular, spatial resolution of SPECT images may prevent accurate evaluation of smaller (<15% of the left ventricle) territories.

The timing of baseline testing is also important. Because an unrevascularized territory may suffer ischemic injury in the early postbypass period, preoperative testing may appear imprecise in predicting improvement in perfusion or recovery of function after surgery. Finally, overall

clinical outcome after surgery, including quality-of-life assessment, is critical to determine benefit, rather than simply the demonstration of improved perfusion or function to a specific myocardial territory.

To date, safety data appear similar across all studies, with no evidence of local or systemic toxicity as assessed by multiple biochemical markers and other assays. Importantly, the overall mortality rate associated with the surgical (epicardial) delivery technique has not been significantly different from that resulting from catheter or intravascular strategies. An important issue in all trials, and especially surgical trials, is the reporting of deaths and complications. Whether a complication is related to therapy should not be determined solely by the principal investigator and should involve physicians not associated with the study.

Problems

- There is a lack of randomized, placebo-controlled trials.
- There are confounding effects of coronary bypass and appropriate attribution of complications.
- The selection of appropriate “targets” for growth factor administration is difficult.

Consensus

- It is appropriate to proceed to randomized phase 2 trials incorporating more sensitive safety and efficacy assays and more careful reporting of safety and efficacy outcomes.
- Sole therapy (without coronary bypass) is likely to provide more meaningful data.
- The “ideal” regional targeting strategy has yet to be determined.
- Determination of benefits of surgical approaches versus catheter-based strategies awaits larger studies.

IX. Emerging Side Effect Profile

Issues

Animal studies and early clinical trials suggested several characteristic toxicities associated with exposure to FGF-2 and VEGF, the 2 most extensively studied growth factors. Renal insufficiency due to membranous nephropathy accompanied by proteinuria⁸⁵ may be the most significant long-term side effect of FGF-2 administration. Although the mechanism of this side effect is not known, it likely is related to FGF-2 deposition in the heparan sulfate-rich glomerular membrane. Another well-documented side effect is severe hypotension associated with both bFGF⁶⁶ and especially VEGF administration owing to nitric oxide release and arteriolar vasodilation.^{65,67} This has proved to be dose limiting in phase 1 trials of both growth factors.

A theoretical concern associated with any angiogenic growth factor administration is the development of plaque angiogenesis that may precipitate plaque growth or destabilization due to broad-spectrum mitogenicity and chemotactic activity, especially toward macrophages. The latter possibility may be particularly relevant given the ability of FGFs and

VEGFs to induce angiogenesis in vasa vasorum⁸⁶ and the association between plaque angiogenesis and its growth^{87–89} and stability.^{90,91}

Other areas of concern include proliferative retinopathy and occult malignancies. Proliferative retinopathy has been associated with the expression and presence of angiogenic growth factors (predominantly VEGF) in the orbital fluid.^{92,93} The role of angiogenesis in tumor growth and metastasis is well documented,⁹⁴ and facilitation of this process may theoretically lead to accelerated primary tumor growth or stimulation of dormant metastases. However, to date, clinical experience with various growth factors has not substantiated these fears.^{46,47,55,65,80,95}

Problems

- Suppression of nitric oxide release (to inhibit hypotension) may inhibit angiogenic response.
- No long-term safety data exist.
- Stimulation of plaque angiogenesis may require careful patient selection and monitoring.

Consensus

- Oncogenic effects are unlikely with short-term dosing, appropriate patient selection, and local drug delivery.

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