

PREVENT III and IV Trials of E2F Decoy: What Have We Learned?

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Michael S. Conte, MD, Boston, MA

Vein Graft Failure: Nature of the Problem

Although endovascular interventions for distal arterial occlusive diseases continue to improve, vein bypass grafting remains a critical limb or life-saving therapy for many patients with advanced atherosclerosis. Autogenous vein, particularly the greater saphenous, has proven to be an effective and versatile arterial substitute. Given the limited available autogenous conduit, the lack of a suitable small-caliber arterial prosthetic, and the frequency of coexistent coronary and peripheral disease, the importance of maintaining long-term patency of vein grafts is evident.¹

Failure of vein bypass grafts directly results in mortality, limb loss, additional interventions, and diminished quality of life. In the lower extremity, where vein graft surveillance is simplified owing to anatomic position, three distinct phases of graft failure are recognized. Early graft occlusion (ie, within 30 days), which occurs in 5 to 10% of cases, is generally ascribed to technical complications but also includes problems intrinsic to the conduit (eg, small diameter or preexisting vein pathology) as well as extrinsic causes (eg, limited outflow, hypercoagulability). Midterm (3–24 month) and late (> 2 years) vein graft failures are most commonly ascribed to the development of fibrotic intimal hyperplasia (IH) and atherosclerotic degeneration, respectively. Rates of reintervention for lower extremity grafts are highest in the mid-term period (20 to 30%), thereby focusing attention on IH as a critical process for therapeutic targeting. It should be noted, however, that there are little data available on the dynamic process of vein graft remodeling in humans. Animal models of vein grafting are largely models of arterialization, as opposed to graft failure. Little, if anything, is known about patient-specific risk factors. Differences in graft remodeling between patients or, more pointedly, at different sites within the same conduit are not understood. Accordingly, the therapeutic approaches under consideration have been based largely on preclinical data and hypothetical mechanisms that remain unproven in humans. This situation is analogous to the level of understanding of angioplasty restenosis a decade ago.

The cell and molecular biology of IH have been best characterized in the context of acute arterial injury such as balloon angioplasty. SMC proliferation has been considered a critical target for the therapy of IH in settings of vascular injury. Many cytokines and growth factors have been identified to influence SMC proliferation, but ultimately these pathways converge on the cell cycle. An increasing understanding of cell cycle regulation has led to the identification of a number of specific molecular targets that may inhibit progression to mitosis. A family of transcription factors (proteins that function to regulate the expression of specific genes) known as E2F is involved in the control of multiple cell cycle regulatory genes, playing a critical role in the progression of cells to division. Molecular approaches targeting cell cycle proteins such as E2F have been developed as strategies for neoplastic and vascular proliferative disorders.

Genetic Modification of Vein Bypass Grafts: Rationale

The attraction of a genetic approach to vein graft failure is based on the concept that the tissue at risk (ie, the vein) is immediately available prior to the onset of the pathologic process and the hope that a genetic reprogramming of cells in the vein wall can lead to an improved healing response.² Vein grafts are uniquely amenable to genetic manipulation because the delivery of genetic material can be achieved under carefully controlled, ex vivo conditions that favor both safety and efficiency. Safe delivery of a fully active transcriptional unit (gene) to a majority of cells within the vein graft wall is a significant hurdle, particularly within the temporal constraints of an intraoperative setting. Though considerable progress has been made in recent years and clinically usable systems are already available, further development of vector technology will be required to achieve the ideal system for intraoperative delivery of intact genes to vein grafts. Another approach to genetic manipulation, which does not require the efficient transfer of large, intact genes, is gene inhibition. A specific gene, or an entire cellular program (eg, cell cycle), may be inhibited using small nucleic acid molecules—oligonucleotides—that may function to either block transcription (“antisense”) or block the activity of critical transcription factors that control gene expression. The latter strategy involves the design of small, double-stranded deoxyribonucleic acid (DNA) molecules (ODN) that serve as a “decoy” for the transcription factor, preventing it from interacting with its normal sequence target in the cell’s chromosomal DNA.³ Small oligonucleotides in solution may be delivered far more easily to cells and tissues with high efficiency and do not require specific viral or nonviral vectors. The use of nondistending pressure has been shown to result in a > 80% uptake of ODN by cells within the saphenous vein wall within 10 minutes of exposure.⁴

A decoy approach targeting cell proliferation was recently developed and tested in animal models of arterial injury and vein bypass grafting.^{5–7} A double-stranded oligodeoxynucleotide (14 base pairs) was designed to incorporate the binding site for the transcription factor E2F, which controls the expression of multiple genes that are responsible for progression of the cell cycle in proliferating cells. Vein grafts treated with the E2F decoy in solution at the time of implantation demonstrated marked inhibition in intimal hyperplasia and resistance to graft atherosclerosis for up to 6 months in cholesterol-fed rabbits.

PREVENT: Clinical Trials of Edifoligide (E2F Decoy)

The E2F decoy strategy for preventing vein graft failure has now been examined in a series of clinical trials known as the Project of Ex vivo Vein graft Engineering via Transfection (PREVENT). PREVENT I was a single institution pilot study in patients (N = 41) undergoing lower-extremity vein bypass.⁸ Intraoperatively, the veins were harvested, mounted on a cannula, and inserted into a device for pressure-mediated transfection with ODN. This small study demonstrated the safety and feasibility of intraoperative transfection with the E2F decoy ODN and suggested the possibility of biologic efficacy.

Subsequently, a corporate-sponsored (Corgentech, Inc., Palo Alto, CA) phase II trial (PREVENT II) in patients undergoing coronary artery bypass graft surgery was completed in Germany.⁹ A total of 200 patients were randomized to treatment with E2F decoy or saline control. Follow-up included both clinical events and imaging (angiography and intravascular ultrasound) at 1 year. As in the PREVENT I trial, no adverse events or complications were attributable to decoy ODN treatment. The graft level analysis revealed a 30% relative reduction in critical stenosis (? 75%, p = .03). Analysis of intravascular ultrasound images revealed a statistically significant reduction in total wall volume (30%), suggesting an influence on remodeling throughout the lengths of the treated vessels.

Based on results from the preclinical and early clinical trials noted above, combined with the significant unmet clinical need, the US Food and Drug Administration approved a fast-track designation for a phase III clinical trial program to examine edifoligide for the prevention of vein graft failure. Parallel randomized trials in lower extremity (PREVENT III) and coronary bypass (PREVENT IV) surgery were designed and executed. Both of these studies have recently reported on the initial unblinding of data, though full analyses of other clinical end points and subgroups are still awaited.

PREVENT III: Phase III Trial of Edifoligide (E2F decoy) in Lower-Extremity Vein Bypass

A phase III trial of E2F decoy ODN for the prevention of lower extremity vein graft failure (PREVENT III) was initiated by the corporate sponsors (Corgentech and Bristol Myers Squibb) in November 2001. The study involved a multicenter, randomized, double-blinded, and placebo-controlled design. A total of 1,404 patients requiring autogenous vein bypass for critical limb ischemia (CLI: rest pain, ulceration, or gangrene) were randomized to either E2F decoy or saline delivered via the graft transfection apparatus. The study involved vascular surgeons from a total of 83 sites across North America and was powered to detect a 30% reduction in the primary end point of graft failure at 1 year. Secondary end points included incidences of critical graft stenosis by ultrasonography and recurrent limb ischemia, as well as quality-of-life assessments. Subject enrollment was completed in October 2003, and data unblinding occurred in December 2004. The initial data were presented at the Society for Vascular Surgery annual meeting in June 2005.¹⁰

The PREVENT III trial design was broadly inclusive and therefore serves as an accurate representation of the current population of CLI patients undergoing revascularization procedures. Nearly two-thirds (64%) of the patients had diabetes, 48% had a history of advanced coronary artery disease, 12% were on dialysis, and 28% had previously undergone an infrainguinal bypass procedure. Tissue loss was the presenting symptom in 75% of patients. High-risk conduits were employed in 24% of cases, including alternative vein in 20% (15% spliced, 5% non-GSV) and small-caliber (< 3 mm diameter) grafts in 6%. The majority (65%) of grafts were placed to infrapopliteal targets.

Perioperative (30-day) mortality occurred in 2.7% of patients. Major morbidity included myocardial infarction in 4.7% and early graft occlusion in 4.3% of patients. Ex vivo treatment with edifoligide was well tolerated. There was no significant difference between the treatment groups in the primary or secondary trial end points, primary graft patency, or limb salvage. However, a significant improvement was observed in secondary graft patency at 1 year (81% edifoligide, 76% placebo, p = .0299). The reduction in secondary patency events was manifest within 30 days of surgery (relative risk for 30-day event for edifoligide 0.45; 95% CI 0.27 to 0.76; p = .001). For the overall cohort at 1 year, survival was 84%, primary patency was 60%, primary-assisted patency was 77%, secondary patency was 79%, and limb salvage was 88%. Given the medical and technical complexity of the study cohort, the outcomes in PREVENT III measure up quite well to the standards expected from retrospective, single-center reports.

The primary results of PREVENT III demonstrate conclusively that a single, ex vivo treatment of lower extremity vein grafts with edifoligide did not confer protection from reintervention for graft failure. However, although none of the prespecified study end points were met, a significant improvement in secondary patency was observed. The risk reduction observed, although modest (4.7% absolute, 19.7% relative reduction in secondary patency events), is not clinically irrelevant and appears to suggest some beneficial biologic effect that merits further inspection. Ongoing analyses from the database, including subgroup event rates and ultrasound findings, seek to better define the potential source of this secondary patency benefit.

PREVENT IV: Phase III Trial in CABG

The PREVENT IV investigators randomized a total of 3,014 patients at 107 sites. The primary end point was the incidence of critical graft stenosis (> 75%) by angiography at 12 months, which was performed in 2,400 (80%) of patients. Data were unblinded in March 2005. Surgery was considered urgent in 48% of cases, with 43% having either left main or three-vessel disease. There were no significant differences in baseline variables or perioperative events between the treatment groups. There was no difference in the occurrence of the primary trial end point (45.2% versus 46.3%) between treatment groups. Other secondary end points including the incidence of total graft occlusion, minimal graft lumen diameter, and major adverse cardiac events were also not different. At this writing, further details of the PREVENT IV study have yet to be presented or reported.

Conclusions: What Have We Learned and Where Do We Go from Here?

In the wake of the sobering, negative results of these two large-scale phase III studies, the sponsor has suspended any further clinical development for edifoligide. Yet many questions remain unanswered regarding this approach, including an explanation for the beneficial secondary patency effects observed in PREVENT III. Possible explanations for the observed effect include previously unknown antithrombotic or antiinflammatory properties of the drug. It is also possible that the secondary patency benefit relates to a reduction in the virulence of the hyperplastic process, which in its most severe form could result in rapidly progressive or unheralded occlusions. Prevention of amputation or graft reintervention was rightfully selected as the primary study end point in PREVENT III since it directly relates to patient morbidity. However, these end points very likely do not accurately reflect the overall burden of proliferative disease within the graft since a very focal lesion treatable by patch angioplasty and a long severe stricture requiring extensive graft replacement are counted equally as end points. Both studies (PREVENT III and IV) lacked direct quantitative measures of vein graft wall thickness and remodeling. Prior studies in coronary bypass have employed intravascular ultrasonography (IVUS) for this purpose; however, the risk and costs associated with IVUS in large scale clinical trials are a major issue. Furthermore, techniques for quantitating wall thickness in peripheral vein grafts have not as yet been well developed. Lacking a direct measure of graft intimal hyperplasia in these trials, we are ultimately unable to discriminate between an incorrect target (E2F specifically or SMC proliferation in general) or ineffectiveness of the therapy as an explanation for the lack of benefit in preventing vein graft failure. This represents the greatest challenge for future trials.

Nonetheless, much has been learned from the execution of these landmark studies and we anticipate that ongoing analyses of the PREVENT databases will continue to provide important insights into vein graft disease and the care of patients with advanced atherosclerosis in general. For example, the analysis of the use of medical therapies in PREVENT III has quantified the extensiveness of undertreatment of the PAD population with proven medical therapies.¹¹ The trials also clearly demonstrate the need for more clinical and basic research into the process of vein graft remodeling in humans. Despite their negative results, the PREVENT trials represent an important first step in the use of intraoperative genetic therapies to manipulate the vascular healing response.

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