Advances in Gene Therapy

Brian H. Annex, MD, a pioneer in angiogenesis research, discusses the current state of this investigative field, methods for delivery of agents, and areas of ongoing research, including a very recent genetic study in the area of peripheral arterial disease.

Endovascular Today: When did you and your colleagues begin researching genetic links to peripheral arterial disease (PAD)?

Dr. Annex: We have long been interested in the possibility that there are genetic connections to PAD. From a clinical perspective, we believed genetics likely played a role in the development and progression of this disease. Advances in genetics research over the past decades have brought us insights into the pathogenesis of possible new treatments for a number of cardiovascular diseases, and PAD was actually a step or two behind other diseases. What became very clear as we integrated both preclinical and clinical studies is that there are situations in which hemodynamic measures of PAD did not correlate with clinical manifestations. We began looking into this area, and clinical and preclinical findings led to the recent report in which we have identified a quantitative trait in PAD that is guiding our steps as we move forward.

Endovascular Today: How has the field of therapeutic angiogenesis evolved in the last decade?

Dr. Annex: The field of therapeutic angiogenesis is very much alive. As I have said at some presentations over the past couple of years, I do not think the field was ever quite as good as it was touted to be, but it was never quite as bad as some people portrayed it. Over the past decade, we have learned a great deal from the field of therapeutic genesis as applied to PAD. This information is directly related to the field of therapeutic angiogenesis, but in fact just applying the concept has led to a number of investigators exploring this area; I am a strong believer that the more people you have involved in a field of study, the more varied the resultant opinions and findings will be.

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Within the field of therapeutic angiogenesis, there have been a number of very exciting findings in the field within the past few years, particularly in the area of critical limb ischemia (CLI), in which a number of abstracts have been presented with very encouraging results. We are seeing some of this work make its way into manuscript form. Some of these results may be published soon. We are seeing larger trials being planned and initiated, and hopefully there will be some more definitive studies completed in the next few years.

Endovascular Today: How would you describe the progress that has been made in studying and delivering vascular endothelial growth factors (VEGF)?

Dr. Annex: Angiogenic growth factors include a broad array of proteins that can promote endothelial blood vessel growth. It is quite clear that we are making a number of advances in regard to VEGF, which is one of the most extensively studied angiogenic growth factors. It is important to understand that VEGF is part of a very complicated system with many different proteins (referred to as *ligands*) that can bind to a number of different receptors. We are just beginning to appreciate the complexity of this.

For angiogenesis, studies are underway in which we are no longer being forced to give one single form of vascular endothelial factor. New approaches allow us to move

upstream to regulate many related forms of VEGF simultaneously. Two of these studies in particular are worth noting: one is currently being conducted by Sangamo BioSciences (Richmond, CA), where the researchers are employing a DNA-binding VEGF-activating transcription factor that has the ability of turning on multiple isoforms of VEGF. The second is by Genzyme Corporation (Cambridge, MA), which is studying hypoxia-inducible factor-1 alpha (HIF-1 alpha), a transcription factor that will also turn on VEGF expression along with a number of other proteins. Thus, rather than being forced to use a single isoform of VEGF, the goal of this method is to turn on multiple forms of VEGF at the same time.

Many people believe that protein therapy for PAD will be inadequate simply because of the duration of protein expression. That is probably true, but one could argue that there are some protein forms that have the ability to stick in the area that they are delivered. Therefore, the generalities that likely apply to VEGF protein, for example, need not necessarily apply to other proteins. This has been behind the rationale for gene therapy. Regarding the method of using genes for delivery, there are studies using plasma DNA, adenoviral constructs, and even some newer constructs currently under investigation with various viral forms that are likely to enter human studies very soon.

Endovascular Today: How would you describe the means by which these growth factors are physically delivered to the patient?

Dr. Annex: That is the beauty of applying therapeutic angiogenesis for PAD patients. Simply put, it is extremely easy to administer an agent to the leg and its muscle. Therefore, the treating physician has the opportunity to administer multiple injections and deliver repeat injections over time with little to no patient-related problems. This is in marked contrast to when comparable agents are considered for ischemic heart disease, where delivering to the heart multiple times is far more problematic. This is especially the case within the confines of a study. Most PAD approaches have used very simple intramuscular delivery. Some groups feel that there is added benefit from using ultrasound guidance to confirm location in the muscle. There is also the potential to deliver growth factors along the vessel wall using imaging.

This ease of delivery has its downsides. Because we are delivering these agents to the lower leg, and it is relatively easy to do, this allows heterogeneity in trial designs. For example, some theories state that one should target therapies to particular areas, such as in and around the area of occlusion or only distal to an area of occlusion. Unfortunately, most of these concepts are theoretical and not based on data, which is a challenge as we move forward into human studies. The more combinations of dose, frequency, and location that are available, the more complex the human study will be.

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Endovascular Today: Recent research at Duke University has identified a genetic mutation linked to PAD in mice. What can you tell us about that finding?

Dr. Annex: We have just identified a region of the mouse genome that affects the degree of recovery that occurs after the induction of hind limb ischemia. This study was based on clinical findings. For example, two patients can have virtually the same risk factors, nearly identical hemodynamic measures, and in some cases, even identical angiographic studies. Yet, one patient will have the clinical manifestation of intermittent claudication, which is pain when walking that is relieved with rest, and the other will have CLI, which is characterized by tissue loss.

Using genetically different strains of mice, we found that one strain nearly completely recovers after surgical repair of hind limb ischemia, while another strain goes on to have a high incidence of tissue loss. The mice underwent the exact same surgical procedure. We then used a series of classic genetic approaches to localize what accounted for the differences. Then, using a number of measures, we identified a region on mouse chromosome 7 to be clearly linked to the ability of the mouse to avoid tissue loss.

We then went on to another model of injury—a wound-healing model—and in that model, the locus on chromosome 7 had no effect. Therefore, the genetic locus related to hind limb ischemia. We are excited about this finding and have initiated work in multiple directions. First we will try to further refine the genes within that locus. We are working on understanding exactly what those genes are—some of them are known, and some of them are unknown.

Finally, we are planning human investigations to follow up on the mouse studies. The information that we have to date is a start. The area of genetics is extremely powerful, and I think it has a lot of potential for advancing our understanding of PAD. Our study is the first of its kind that relates not to the generation of PAD, but to the clinical manifestations that follow.

Endovascular Today: If similar findings were developed in a human study, how could researchers and clinicians use these genetic biomarkers to identify and potentially offer new therapies?

Dr. Annex: I think the potential for the results to advance human study are quite real. The data suggest that genes influence the clinical manifestations of PAD. It therefore simply stands to follow that if a genetic influence is strong enough to influence the clinical manifestations, these findings may have an impact to find pharmaceutical and novel therapies. To some degree, as vascular medicine specialists, we have approached PAD as one disease, which is clearly the correct thing to do in that all the patients suffer from atherosclerosis and its sequelae; but our findings suggest that these genetic differences may have a profound impact on the long-term clinical manifestations. It follows that these genes have the potential to have an impact on therapies, including angiogenesis. When we started the genetic studies, we were hoping that there would be an angiogenesis in that group, and much to our disappointment, there was not an angiogenic gene. As we are beginning to dive into the data a little more deeply, we are starting to see how some of the proteins may relate to angiogenesis itself.

Endovascular Today: Do you believe that therapeutic angiogenesis will become a mainstream method of therapy?

Dr. Annex: I truly believe that therapeutic angiogenesis has a future. There is clear evidence suggesting that we are going in the right direction. However, I will still question whether we are absolutely targeting the ideal study or the best population. The potential for angiogenetic therapy to be an adjuvant to other forms of treatment for PAD may be as great or greater than its ability to be used as a stand-alone agent. Unfortunately, it does require different study designs, and thus far, few people have been willing to undertake that challenge.

For stand-alone therapy studies, a number of agents have already entered and completed phase 2 trials. How quickly an agent will move from phase 2 until it is available for clinical use is highly variable and very much dependent on the agent, its therapeutic benefit, and its potential toxicities. Ultimately, the final decision is one that is made by the FDA, and I think it is made on a case-by-case basis using standard agency guidelines. In summary, it is remotely possible that because PAD is such a challenging disease with such negative clinical outcomes, one could imagine a situation in which a particular agent could be approved within a 3- to 5-year period as opposed to a 7- to 10-year period, if convincing data were derived from a phase 3 study. Any such approval would also depend on the agent, its therapeutic efficacy, and a more thorough study to document the absence of toxicities.

Endovascular Today: Do you predict that angiogenesis will be more successful in certain cases versus others, certain types of anatomies, and certain degrees of PAD?

Dr. Annex: There is a belief in cardiovascular medicine that, in general, the sicker the patient is, the greater the potential gain will be. Because CLI is certainly the most severe manifestation and has the most ability to show benefit, I believe CLI will be the area that meets with success first. However, studies in CLI subjects are extremely complicated and are difficult to carry out. There is a fair amount of patient heterogeneity. As one thinks about an agent, additional variables include dose, route, and frequency. In addition, one has to consider the potency of an agent. I like to use the analogy of the hammer and the nail. Rubber mallets work relatively well on rubber nails and not necessarily very well on very hard nails or big nails. Therefore, I think that the target for any agent really needs to be carefully considered in relation to the potency of the agent and the potential downstream toxicities.

Endovascular Today: If therapeutic angiogenesis is shown to be an effective treatment modality, is there a particular interventional specialty that would be best suited to deliver this type of therapy?

Dr. Annex: I think the care of patients with PAD and vascular disease in general needs to move forward, and I worry less about whether it is best within the hands of vascular medicine specialists, cardiologists, cardiologists with vascular medicine training, interventional radiologists, or vascular surgeons. At the very best centers, we are seeing a blurring of those lines as therapy is moved forward in more of a team-oriented effort. There are elements that every specialty has to offer, and I see no obvious reason that any group would be particularly favored or disadvantaged as this therapy moves forward. ■

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