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Integrin $\alpha_v\beta_3$ as a Target in the Prevention of Neointimal Hyperplasia

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Abstract

While there are major advances made in the treatment of recurrent stenosis (restenosis) often resulting from percutaneous coronary and peripheral interventions, the persistent complications of acute thrombosis secondary to intimal hyperplasia and restenosis remain a mainstay for repeat hospitalizations in this patient population. For many years, a ubiquitous cell surface receptor called the $\alpha_v\beta_3$ integrin was the target of many investigators in the prevention of intimal hyperplasia and restenosis as its interaction with the extracellular matrix was believed to coordinate the migration of smooth muscle cells from the media to the intima, the seminal event in the formation of intimal lesion. After the publication of uniformly positive studies demonstrating that $\alpha_v\beta_3$ integrin blockade led to a significant reduction in new intimal (neointimal) lesion formation in a variety of animal models of balloon angioplasty, early clinical trials supported the association of decreased target lesion revascularization and the use of antagonists to the SMC integrin $\alpha_v\beta_3$ and its related platelet integrin $\alpha_{IIb}\beta_3$. However, a series of clinical trials subsequently demonstrated that these antagonists did not necessarily prevent revascularizations by inhibiting intimal hyperplasia. Additional animal studies subsequently showed that indeed in the setting of pre-existing smooth muscle cells in the intimal lesion (i.e., atherosclerotic plaque, fatty streaks), inhibiting smooth muscle cell migration by way of β_3 integrin blockade was an ineffective approach in the prevention of intimal hyperplasia and restenosis as demonstrated in the clinical trials. However, given the wealth of basic and clinical information on the $\alpha_v\beta_3$ integrin and the use of its antagonists in the vasculature, we discuss in this manuscript our new approach to an old solution by targeting a new clinical problem of early failure arteriovenous access for hemodialysis. Given the uniqueness of arteriovenous access in that there are essentially no significant atherosclerotic lesions in the artery and vein prior to the anastomosis, the seminal event of the coordinated migration of smooth muscle cells from the media to the neointima could by targeted once again with β_3 integrin antagonists.

Introduction

While there are significant advances made in the primary (e.g., surgical bypass and angioplasty and stenting) and secondary treatments (e.g., drug-eluding stents) for coronary and peripheral arterial occlusive disease, the ultimate solution to the persistent problems of anastomotic and in-stent narrowing (or restenosis) and the resulting acute thrombosis remains elusive.[1,2] Restenosis is the reduction of the arterial luminal size due to loss in lumen size following the percutaneous and open arterial intervention, and its pathogenesis is thought to be multifactoral

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with a complex orchestrating of a number of biochemical and cellular events.[3,4] The initial response to injury of the arterial wall during the formation of an anastomosis or overstretching by balloon catheter is elastic recoil, responsible for loss of initial luminal gain (constrictive remodeling), which characterizes the early and late phases of restenosis. The endothelial disruption and the exposure of subintimal components initiate the middle phase with platelet adherence and aggregation, fibrinogen binding, and thrombus formation. The thrombus, in turn, creates a scaffold into which smooth muscle cells (SMC) can migrate, synthesize matrix, and reorganize the thrombus, providing the substrate for intimal growth or intimal hyperplasia. Moreover, inflammatory mediators and cellular elements contribute to trigger a complex array of events that modulate matrix production and intimal cellular proliferation. The present manuscript focuses on the $\alpha_v\beta_3$ integrin, a cell surface receptor, as a potential therapeutic target for the prevention of SMC migration and restenosis.

$\alpha_v\beta_3$ integrin structure, function and distribution

Integrins are a family of transmembrane glycoproteins that mediate cell-cell and cell-matrix interaction.[5] All known members of this superfamily are noncovalently associated heterodimers composed of an α and a β subunit. At present, at least 8 β and 18 α subunits have been characterized, and these subunits associate to generate at least 24 different integrins.[5] For instance, subunit β_3 associates with subunits α_{IIb} and α_v to generate integrins $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$. Integrins are type I membrane proteins with a large extracellular, a transmembrane and a short cytoplasmic domains. The interaction between integrins and their ligands, besides mediating cell adhesion, plays a role in a number of cellular processes.[6]

$\alpha_v\beta_3$ integrin is one of the most prevalent integrins - expressed on almost all the cells originating from the mesenchyme and on a variety of cell types in the blood vessel (e.g., endothelial cells, SMCs, fibroblasts, macrophage, and platelets). It is known to mediate many biological events (e.g., migration of vascular SMCs, adhesion of osteoclasts to the bone matrix and angiogenesis). It is the most promiscuous integrin for it binds to many different ligands including a number of extracellular matrix proteins (e.g., vitronectin, fibronectin, osteopontin, fibrinogen and von Willebrand factor) via the interaction with the Arg-Gly-Asp (RGD) motif.[5,7] On the other hand, a related integrin, $\alpha_{IIb}\beta_3$ is exclusively expressed on platelets and is largely responsible for the final cohesive phase of platelet activation *in vivo*, such as platelet aggregation supported by the binding of adhesive protein.[8] Interestingly, the $\alpha_{IIb}\beta_3$ integrin recognizes the same RGD motif and binds to the same extracellular matrix proteins.[9,10]

Osteopontin (OPN), one of the ligands for $\alpha_v\beta_3$, contains the canonical integrin recognition sequence, RGD and bind to $\alpha_v\beta_3$ integrin through the sequence.[11] *In vitro* studies have demonstrated that OPN promotes the migration of cultured rat arterial SMC[12] and human coronary artery SMC.[13] Previous data showed that OPN was coordinately expressed with β_3 integrins in the vessel wall and that a blockade of $\alpha_v\beta_3$ resulted in a reduction of neointimal formation in animal models following vascular injury.[14] These data suggest that $\alpha_v\beta_3$ binding to OPN are important in mediating SMC migration from the media to the neointima *in vivo*.

The integrin-mediated adhesion of cells to extracellular matrix leads bidirectional intracellular signaling events that regulate cell migration, as well as survival and proliferation. In outside-in signaling, ligand binding activates intracellular signaling pathways. In inside-out signaling, signals received by other receptors activate intracellular signaling pathways that impinge on integrin cytoplasmic domains, and change the extracellular domain conformation for binding to ligands.[5] Recent studies have shown that $\alpha_v\beta_3$ expression on SMC is subject to regulation and is increased by treatment with thrombin,[15] transforming growth factor-β (TGF-β) and platelet-derived growth factor-BB (PDGF-BB).[16] In endothelial cells, vascular endothelial growth factor (VEGF) can induce activation of $\alpha_v\beta_3$ and NF-κB3 which leads to suppression

of p53 and p21WAF1/CIP1 is an important transcription factor in $\alpha_v\beta_3$-dependent signals for endothelial cells.[17,18] Moreover $\alpha_v\beta_3$, along with membrane type 1 matrix metalloproteinase-1 (MT1-MMP), is associated with matrix metalloproteinase-2 (MMP-2) at the cell surface.[19,20]

MMPs belong to a family of zinc-dependent endopeptidases that degrade many components of the extracellular matrix. Most MMPs are secreted in a latent form (pro-MMP), and a specific multistep activation process is required to convert pro-MMP to proteolytic active forms. Localization of functionally active MMP on the cell surface is essential and tightly regulated elements during a variety of normal and disease processes, such as tumor cell invasion.[21] For instance, MMP-2 is activated at the cell surface of invasive cells by a multimeric receptor/activation complex consisting of the tissue inhibitor of metalloproteinase 2 (TIMP2), and the membrane type 1 MMP (MT1-MMP).[22] In line with the theory of cellular invasion requiring a coordinated expression of proteolytic enzymes and adhesion molecules, Hofmann and colleagues[23] suggested that functional cooperation of MT1-MMP and $\alpha_v\beta_3$ is critical for spatial and temporal control of extracellular matrix proteolysis in human melanoma cells. They indicated that joint MT1-MMP and $\alpha_v\beta_3$ might enforce most efficient docking, and activation of MMP-2 and, in turn, facilitate cellular locomotion. Furthermore, Brooks and colleagues[19] demonstrated that the functionally active form of MMP-2 on the cell surface seems to predominantly involve $\alpha_v\beta_3$ in angiogenesis and concomitant melanoma growth.

$\alpha_v\beta_3$ integrin and animal models of arterial injury

Our group first reported the potential therapeutic benefit of $\alpha_v\beta_3$ blockade in the prevention of intimal hyperplasia and restenosis.[24] We demonstrated that a potent chemotactic agent present in the arterial wall following injury, PDGF, regulates the surface distribution of $\alpha_v\beta_3$ on the SMC surface in cell culture. Using indirect immunofluorescence, focal adhesions containing $\alpha_v\beta_3$ were localized to the leading edge of migrating cells when stimulated with PDGF. In contrast, $\alpha_v\beta_3$ was evenly distributed on the surface of SMC grown in the absence of PDGF. These results suggest that a redistribution of $\alpha_v\beta_3$ in focal adhesion is necessary for SMC motility.

In an *in vitro* assay, we determined that PDGF-induced human SMC migration is mediated by $\alpha_v\beta_3$ by using a blocking antibody to $\alpha_v\beta_3$ (LM609). This PDGF-mediated migration was also attenuated with an $\alpha_v\beta_3$-blocking RGD peptide (GpenGRGDSPCA) demonstrating that the RGD sequence is the binding site in the extracellular matrix proteins. We also tested the effects of the local administration of this RGD peptide in a rabbit model of carotid balloon angioplasty injury. This RGD antagonist was delivered to the adventitia of the injured artery and inhibited the new intimal (neointimal) lesion formation by 70%. Neointimal hyperplasia seen in an animal model should be distinguished from intimal hyperplasia seen in humans as there are no inherent SMC in the non-injured intimal layer in most, normocholesterolemic animals. Subsequently, the same peptide locally applied to the carotid artery through an adventitial pluronic gel in rats led to a 92% reduction in neointimal hyperplasia after a similar balloon angioplasty injury.[25]

$\alpha_v\beta_3$ is present both in normal artery and in site of SMC accumulation and angiogenesis in atherosclerotic plaques in humans.[26] In normal artery, $\alpha_v\beta_3$ is generally detectable only along the luminal surface with minimal expression in the media.[26,27] Several studies in animal models have shown that arterial injury is a stimulus for expression of $\alpha_v\beta_3$ by endothelial cells and medial SMC.[24,27] For instance, Srivatsa and colleagues[28] showed in the pig coronary stent model that there is early upregulation of $\alpha_v\beta_3$ at sites of cell accumulation within the neointima and adventitia at 7 days after arterial injury, followed by persistent high levels of $\alpha_v\beta_3$ expression within the media and neointima up to 21 days, decreasing towards baseline

by 28 days. Indeed, Table 1 depicts a number of reports demonstrating the efficacy of $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ antagonists in the reduction of neointimal hyperplasia in a variety of species.

$\alpha_v\beta_3$ integrin and the clinical trials of restenosis

The results from the animal studies were consistent with findings from the early clinical trials examining the effect of various antagonists to platelet integrin $\alpha_{IIb}\beta_3$ and SMC integrin $\alpha_v\beta_3$ on the issue of long-term benefit of reduced target lesion revascularization (Table 2). In the Evaluation of Platelet IIb/IIIa Inhibition for Prevention of Ischemic Complications (EPIC) trial, ReoPro (abciximab, an monoclonal antibody fragment directed against the β_3 integrin) was effective in limiting the need for late coronary revascularization after coronary angioplasty for at least 3 years after treatment.[29] A subsequent study confirmed that ReoPro treatment reduced ischemic complications and late mortality, particularly in the diabetic population.[30]

However, the Integrilin to Minimize Platelet Aggregation and Coronary Thrombosis (IMPACT) II trial, which used Integrilin (an agent with anti-$\alpha_{IIb}\beta_3$ activity but without specific $\alpha_v\beta_3$ inhibitory activity) was ineffective in the reduction of coronary revascularizations in the same clinical setting as the EPIC trial.[31] As in the animal studies, these clinical results suggest that $\alpha_{IIb}\beta_3$ integrin inhibition had no place in the treatment of coronary restenosis. However, a more detailed clinical study (ERASER trial) revealed that ReoPro given at the time of or a short duration after coronary angioplasty and stenting had little or no effect on the size of the intimal hyperplastic lesion as measured by intravascular ultrasound.[32] Still, these clinical trials did not adequately address the role of $\alpha_v\beta_3$ in restenosis since short-term infusions of Integrilin and ReoPro would not be expected to block $\alpha_v\beta_3$ during crucial periods of vascular repair. Bleeding complications limited the long-term administration of these antagonists during percutaneous intervention to humans. Indeed, there is no certainty the local concentrations of these antagonists in the vessel wall are sufficient to inhibit $\alpha_v\beta_3$ integrin clinically.

$\alpha_v\beta_3$ integrin and the clinical significance based on animal models

As the exact role of $\alpha_v\beta_3$ in intimal hyperplastic lesion formation and restenosis remains unknown, it became critical to re-examine the precise mechanism of action of $\alpha_v\beta_3$ in cell culture and in animal models. Indeed, Azrin and colleagues[33] tested in a hypercholesterolemic rabbit model of balloon angioplasty with pre-existing atherosclerotic lesions an antibody (AZ-1) that binds to the rabbit platelet $\alpha_{IIb}\beta_3$ and inhibits platelet function *in vivo*. There were no significant differences in intimal hyperplastic lesion formation between the AZ-1 antibody-treated and control groups 4 weeks after angioplasty. In this case, the $\alpha_{IIb}\beta_3$ antagonist failed to inhibit restenosis in the setting of pre-existing intimal lesion, similar to the human clinical situation where SMC migration is not a requisite for intimal lesion generation. While the case could made simply against the $\alpha_{IIb}\beta_3$ antagonists in the treatment of intimal hyperplasia, Deitch and coworkers[34] reported that ReoPro failed to reduce the intimal hyperplastic lesion formation in atherosclerotic nonhuman primates after angioplasty and stenting in separate arteries, suggesting that in the setting of pre-existing intimal SMC, blockade of SMC migration is not critical.

Since the publication of the reports on the ineffectiveness of $\alpha_v\beta_3$ blockade on the intimal hyperplasia development in complex animal models, Smyth and colleague[35] used a combination of “guidewire-induced endothelial denudation and arterial ligation” and demonstrated that (β_3-integrin deficiency ($\beta_3^{-/-}$) did not have a role in intimal lesion formation.

However, our group subsequently classified the injury methodology by creating 3 distinct injury patterns that differed in the extent of medial injury induced in these $\beta_3^{-/-}$ mice: (1) guidewire probe–induced transmural injury with medial disruption; (2) nonmedial disruptive ligation injury; and (3) eccentric medial disruptive injury followed by arterial ligation.[36] We

believed that guidewire probe injury generated more transmural mechanical damage to the media over a longer segment of the vessel compared with the ligation injury, which generates a more modest, focal lesion with stagnant flow and thrombosis.

As before, we showed that β_3-integrin deficiency did not protect against neointimal lesion formation after a significant medial disruption seen with guidewire probe injury. In contrast, in the setting of arterial ligation injury, β_3-integrin deficiency protected against neointimal lesion formation at 1, 2, and 3 weeks and 3 months after injury. When the combination of medial disruption and arterial ligation was used in $\beta_3^{-/-}$ mice, there was eccentric neointimal lesion formation only at the site of disruption. The lack of neointimal lesion formation on the opposite, nondisrupted section is consistent with the dependence of neointimal formation on the mechanical disruption of the internal elastic lamina and media as described by others.[37]

One satisfactory explanation for these discrepancies based on the arterial injury patterns in $\beta_3^{-/-}$ mice is that different models and/or methodologies accentuate the various, distinct functions of β_3 integrins. For instance, Carmeliet and colleagues[38] compared mechanical injury–induced intima formation in plasminogen activator inhibitor 1 (PAI-1)–deficient and wild-type mice and demonstrated that PAI-1 blocks intimal thickening by inhibiting the migration of SMCs. In contrast, Peng and colleagues[39] demonstrated that when ligation-induced intima formation was examined in PAI-$1^{+/+}$ and PAI-$1^{-/-}$ mice, PAI-1 promoted neointimal thickening. One can conclude that these injury models emphasize a contrasting cascade of events despite the apparently simple injuries. Moreover, Tanaka and colleagues[40] showed in a carotid ligation injury model, there is minimal bone marrow-derived cell contribution to the neointimal lesion development. Therefore, in the carotid ligation model of intimal hyperplasia, the seminal event appears to be a directional cellular migration from the media to the neointima with the aid of $\alpha_v\beta_3$ with little or no contribution from the bone marrow (Figure 1B). Hence, although β_3-integrin blockade effectively reduces neointimal hyperplasia in animal models, this blockade may not be effective for prevention of neointimal lesion formation in the less defined, more disruptive injury induced by percutaneous transluminal coronary angioplasty in human coronary arteries (Figure 1A).

Future directions

The complications with the hemodialysis access constitute a major cause of morbidity for patients with end-stage kidney disease. In the United States alone, approximately 70% of the 250,000 patients on hemodialysis use expanded polytetrafluroethylene (ePTFE) grafts for permanent vascular access.[41] Currently, the 1 and 2-year primary patency rates of these ePTFE grafts are 50% and 25%, respectively, while hemodialysis access related hospitalizations cost well over 1 billion dollars per annum.[42] The failure of hemodialysis access grafts is predominantly due to a neointimal hyperplastic response in the region of the venous anastomosis resulting in reduction of shunt flow and ineffective hemodialysis. Subsequently, the access needs to be revised by an open surgical revision or a percutaneous angioplasty and/or stenting. By then, placement of another hemodialysis access at a difference site is not far off quickly exhausting the available sites predominantly in the upper extremities.

Castier and colleagues[43] created recently an arteriovenous fistula (AVF) model in mice that demonstrated a rapid neointimal hyperplasia development at the anastomosis, the site most relevant to the clinical problem of venous neointimal hyperplasia and acute thrombosis. In this AVF model, there are traumatic injury to the blood vessels involved and turbulent blood flow near the anastomosis along with a compliance mismatch between artery and vein that believed to be factors that produce the rapid neointimal lesion formation. They further demonstrated that like the arterial ligation injury model, the neointimal SMC of the AVF anastomosis do not originate from bone marrow stem cells. Hence, they have demonstrated that this animal model

and the clinical situation of arteriovenous access surgery for hemodialysis are uniquely suited to target the seminal event in the formation of the neointimal lesion formation, the SMC migration (Figure 1C). Unlike intimal hyperplasia seen with preocclusive atherosclerotic arteries after angioplasty and stenting, neointimal hyperplasia is seen with an anastomosis involving a synthetic graft (e.g., ePTFE, Dacron) and a relatively disease-free segment of vein or artery. Hence, there is no pre-procedural, stenotic intimal plaque with abundant resident SMCs. Therefore, adhesion and directional migration (relocation) of SMCs into the provisional matrix on the luminal surface are indeed the seminal events, not unlike the invasive tumor cells (metastasis). In this setting of ESKD and AV access, targeting the $\alpha_v\beta_3$ integrin could have a significant impact on the prevention of neointimal hyperplasia. Indeed, animal studies examining the role of the $\alpha_v\beta_3$ antagonists on the long-term patency of the AV accesses need to be performed in the setting of uremia, and then perhaps properly designed clinical trials in this ESKD patient population might ultimately provide a clinical problem to this old solution.

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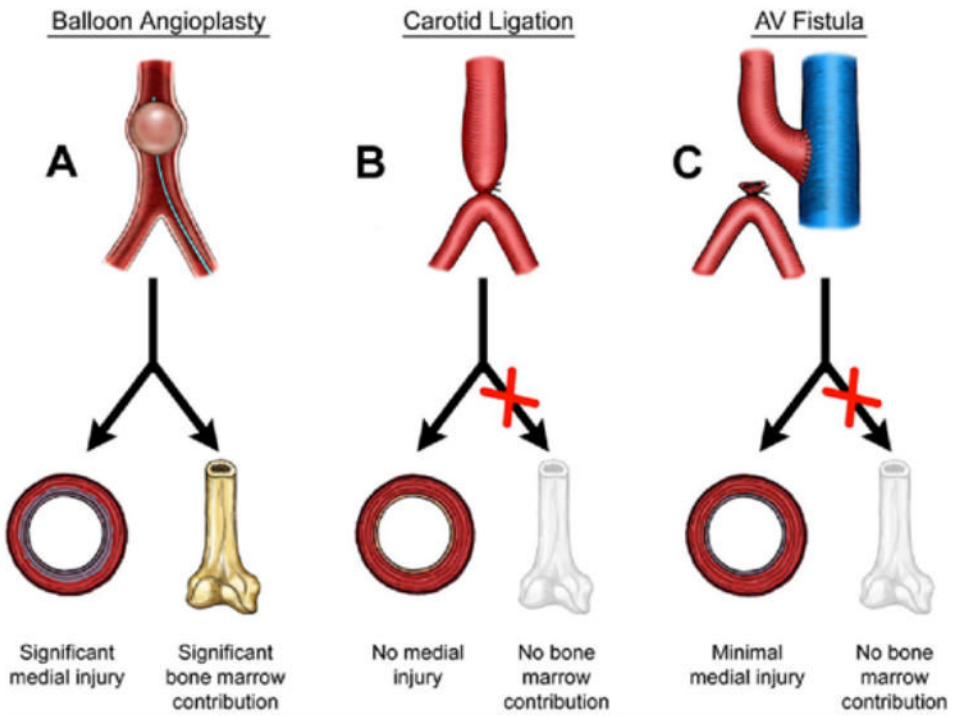

Figure 1.
Leading animal models of arterial injury. A. The balloon angioplasty model results in a disruptive injury to the intima and media leading to neointimal hyperplastic lesion formation with a significant SMC contribution from the bone marrow. B. The carotid ligation (flow cessation) model results in little or no disruptive injury to the media. The seminal event appears to be a directional SMC migration from the media to the neointima with little or no contribution from the bone marrow. C. Like the carotid ligation model, the critical event appears to be SMC migration with minimal or no medial disruption and bone marrow contribution.

Table 1

Scientific investigations on the effectiveness of the β_3 integrin antagonists in the inhibition of intimal lesion formation in various animal models of arterial injury.

Species	Antagonist against	β_3 integrin	Artery	Type of injury	IH lesion reduction?	Reference
Rat	ReoPro	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$	Carotid	Angioplasty	Yes	25,44
Rat	Gpen	$\alpha_v\beta_3$	Carotid	Angioplasty	Yes	45
Hamster	Gpen	$\alpha_v\beta_3$	Carotid	Angioplasty	Yes	46
Hamster	FK633	$\alpha_{IIb}\beta_3$	Carotid	Angioplasty	Yes	47
Rabbit	Gpen	$\alpha_v\beta_3$	Carotid	Angioplasty	Yes	24
Rabbit	Vitaxin	$\alpha_v\beta_3$	Carotid	Angioplasty	Yes	48
Rabbit	AZ-1	$\alpha_{IIb}\beta_3$	Femoral	Angioplasty	No	33
Pig	XJ 735	$\alpha_v\beta_3$	Coronary	Stenting	Yes	28
Monkey	ReoPro	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$	Iliac	Angioplasty	No	34
Monkey	ReoPro	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$	Subclavian	Stenting	No	34

IH (intimal hyperplastic).

Table 2

Clinical trials on the effectiveness of the β_3 integrin antagonists in the inhibition of restenosis in the coronary arteries.

Study	Antagonist against	β_3 integrin	Type of injury	TLR	Reference
EPIC	ReoPro	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$	Angioplasty, atherectomy	Reduced	49
Lincoff et al.	ReoPro	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$	Stenting	Reduced	30
IMPACT II	Integrilin	$\alpha_{IIb}\beta_3$	Angioplasty	No difference	31
ERASER	ReoPro	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$	Stenting	No difference	32
CAPTURE	ReoPro	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$	Angioplasty	No difference	50

TLR (targeted lesion revascularization).