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# Neoatherosclerosis after drug-eluting stent implantation: a novel clinical and therapeutic challenge

by

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**ABSTRACT**

The recognition that obstructive disease of the epicardial coronary arteries, causing ischemic heart disease, can be treated with a percutaneous coronary intervention (PCI) has been a major discovery in cardiology in the last 40 years contributing, in particular, to the reduction of mortality associated to acute myocardial infarction (AMI). However, even in the era of drug-eluting stent (DES) implantation, a sizable proportion of patients who undergo PCI may develop late or very late post-implantation complications, that occur in the form of restenosis, neoatherosclerosis and/or in-stent thrombosis. Such complications are clinically relevant since they can cause AMI and negatively impact on the outcome. The underlying pathophysiological mechanisms are complex but related to inhibition of neointimal proliferation by DES that, on the hand, reduces the rate of in-stent restenosis, but, on the other hand, causes dysfunctional vessel healing, persistent inflammation, platelet activation and adverse immunologic responses. Multiple approaches have been developed or are under evaluation to target DES-related complications including pharmacotherapy, procedure-related imaging methods, novel stent designs and drug-delivery methods.

The aim of this review is to provide an update on the latest preclinical, translational and clinical pharmacotherapeutic developments in this setting that target novel cellular mechanisms and pathways that might contribute to neoatherosclerosis. Due to the importance of secondary prevention in the reduction of DES-associated complications, this review also provides a short overview of pharmacologic agents that are established or currently being investigated in this regard.

***Keywords:*** *coronary restenosis; coronary thrombosis; drug-eluting stents; neoatherosclerosis; pharmacotherapy; percutaneous coronary intervention;*

## INTRODUCTION

While the introduction of drug-eluting stent (DES) technology for the treatment of significant culprit lesions due to coronary artery disease (CAD) was able to markedly reduce neointimal proliferation, at the same time, a high price was paid in terms of delayed and aberrant arterial healing, due to the effects of anti-proliferative drugs eluted by the stent that impaired physiological reendothelialization and vascular remodeling.[1, 2] Autopsy reports and experiments in animal models also showed that persistent fibrin deposition, chronic inflammation, and continuous platelet activation characterize delayed arterial healing.[3] Most of the current DES implants inhibit the mammalian target of rapamycin (mTOR) that is a member of the phosphatidylinositol 3-kinase-related kinase (PIKK) family of serine/threonine protein kinases.[4, 5] Recently, an interaction between the FKBP12.6 cellular pathway and canonical mTOR inhibitors was discovered as a major cause of vascular permeability and neoatherosclerosis showing that more precise molecular targeting of mTOR complex might ameliorate late complications of interventional treatment such as neoatherosclerosis.[6] Generally, an impaired coronary endothelial function and vasomotion associated to DES implantation is characterized by decreased nitric oxide (NO) production, loose intercellular junctions and decreased levels of antithrombotic mediators, processes that are paralleled by a rapid infiltration, retention, and increased expression of foamy macrophages within the neointima of stented segment thus promoting the formation of a new atheroma.[7] Furthermore, wall shear stress and local hemodynamic forces also play a role in restenosis, thrombosis, and platelet activation after stent implantation.[8]

In the present review, the role of neoatherosclerosis and other late or very late stent complications that occur post-DES implantation are discussed in the context of established risk factors and potential therapeutic targets, with emphasis on novel cellular pathways.

## NEOATHEROSCLEROSIS AS A LATE OR VERY LATE COMPLICATION OF INTERVENTIONAL TREATMENT

### *Definition of neoatherosclerosis, its incidence and incidence of late stent complications*

As the design in stent technology evolved over time, different profiles of clinical complications after stent implantation were observed, corroborated by robust follow-up data. Namely, 1st generation DES implants were able to substantially reduce in-stent restenosis (ISR) events associated with bare-metal stents (BMS) while increasing the incidence of in-stent thrombosis (IST).[9] Notably, ISR occurs earlier in patients implanted with BMS, neointimal hyperplasia (proliferation and migration of vascular smooth muscle cells - VSMC) being the prevalent mechanism.

Furthermore, 2nd generation DES implants were associated with lower rates of IST compared to 1st generation DES, however, a different type of complication leading to late or very late stent failure came to prominence with newer implants, known as "neoatherosclerosis".[10-12] This distinct type of accelerated atherosclerosis of multifactorial etiology develops inside of the stented segment of a coronary vessel.[13-15] Neoatherosclerosis seems to marked by three distinct phases that encompass early infiltration by foamy macrophages, in-stent atherosclerotic plaque development, and formation of necrotic core plaque with a thin fibrous cap.[16] Moreover, among 2nd generation DES implants, differences were observed in terms of tissue characteristics in early (<1 year) and late (>1 year) ISR – former being mediated mainly by neointimal hyperplasia while neotherosclerosis was the dominant mechanism in the latter.[17] Specifically, late ISR was characterized by higher prevalence of lipid-laden neointima, thin-cap fibroatheroma, neovascularization and macrophage infiltration compared to early ISR and it could be inferred that the delayed arterial healing associated with DES implants might predispose to neaotherosclerosis. Late

stent failure as a consequence of neoatherosclerosis occurs more frequently in DES than BMS largely due to stent underexpansion, and nowadays is recognized as a late or very late complication of a coronary interventional treatment.[18-21] This was confirmed by the autopsy-based study that revealed significantly higher incidence of neoatherosclerosis in DES (31%) compared to BMS (16%) with significantly shorter median stent duration in the former.[18]

Moreover, development of neoatherosclerosis in DES implants has been described as a "*late catch-up"* phenomenon due to observation that neointimal growth is highly suppressed during the 1st year after DES implantation but then shows continuous progression accompanied with rapid deposition of lipid-laden macrophages thus acting as a final common pathway of late stent failure.[22] Indeed, an optical coherence tomography (OCT) registry-based study examining very late stent failure mechanisms among early- and new-generation DES revealed that three most common causes of very late DES failure were strut malapposition (34.5%), neoatherosclerosis (27.6%) and uncovered struts (12.1%).[23] A graph showing the percentage of atherosclerotic change and neoatherosclerosis incidence in DES compared to BMS in relation to implant duration in months is provided in **Figure 1**.

***Risk factors associated with neoatheroslerosis***

While series of intracoronary imaging studies encompassing intravascular ultrasound (IVUS) and OCT along with direct histopathologic analyses from biopsy tissue specimens provided a morphological and compositional characterization of neoatherosclerosis, its etiology and pathophysiology remain largely unknown.[11, 18, 24, 25] Some OCT observations suggest that neoatherosclerosis occurs regardless of stent type and is more dependent on focal triggers within the vessel that are involved in the formation of vulnerable lesions.[26, 27] Some relevant differences in pathologic mechanisms between native coronary atherosclerosis and in-stent neoatherosclerosis, as well as representative OCT images, are provided in **Figure 2**.

The dominant hypothesis about the neoatherosclerosis formation is based on the assumption that neointimal proliferation develops *de novo* within the stent, independently of underlying native plaque; however, this notion is based on a *"snapshot"* morphological lesion analysis and has recently been challenged.[28, 29] The pathogenic mechanisms at the basis of neoatherosclerosis might indeed be similar to those involved in native atherosclerosis progression (e.g., endothelial dysfunction, lipid uptake, inflammation, etc.). An elegant study by Taniwaki et al. recently showed a significant association between in-stent neoatherosclerosis and the progression of native coronary atherosclerosis, assessed as change in minimal lumen diameter (MLD) serially measured within matched coronary segments at baseline and 5-year angiographic follow-up.[24] This study showed a significantly greater reduction in MLD in both target and non-target vessel in patients with in-stent neoatherosclerosis than in those without, suggesting a pathogenic link between the two processes. These findings appear even more relevant considering the relatively low incidence of neoatherosclerosis (i.e., 16% of lesions in the study by Taniwaki) and the need for long-term follow-up in these patients.[24] Importantly, the increased presence of neoatherosclerosis is not only associated with individual atherosclerosis risk factors and underlying comorbidities, but largely with non-traditional risk factors of which most are inherent to stent design, anatomical complexity of the lesion (bifurcations, trifurcations, long lesions, small vessels, etc.), local vessel hemodynamics, and PCI-related procedural variables (stent underexpansion, malapposition, fracture, flow-limiting dissection, and similar). An association of neoatherosclerosis with neovascularization and adjacent lipid plaque has also been established.[30] A comprehensive overview of risk factors associated with neoatherosclerosis is provided in **Table 1**.

Hemodynamic alterations within the stented segment of the vessel might also contribute to neoatherosclerosis. Endothelial shear stress (ESS), which is the tangential force generated

by the friction of the flowing blood on the surface of the vessel wall, affects atherosclerotic disease progression in both native and stented coronary arteries.[31] Several studies suggest that low ESS promotes plaque growth and vulnerable plaque formation in humans.[32] As the pathogenic mechanisms at the basis of neoatherosclerosis appear to be similar to those involved in native atherosclerosis progression (e.g., endothelial dysfunction, lipid uptake, inflammation, etc.), it is conceivable that flow dynamics and ESS play an important role in the development of neoatherosclerosis after stent implantation. Papafaklis et al. reported an inverse correlation between ESS and neointimal hyperplasia in BMS and in first-generation DES.[33] An OCT study showed that in-stent neoatherosclerosis occurs more often at the inner curvature and at the outer waist of a bifurcation, which are typically exposed to low and oscillatory shear stress.[34] A virtual-histology intravascular ultrasound (VH-IVUS) study by Bourantas et al. showed a negative correlation between predominant ESS and the percentage of the neointimal necrotic core component, which is indicative of the presence of neaotherosclerosis, in BMS.[35]

Finally, systemic inflammation and allergic inflammation as reactions to a foreign body are biologically important processes that occur after stent implantation and are associated with restenosis, stent thrombosis and degree of neointimal lesion burden.[36-39]

***Pathophysiology of neoatherosclerosis and its clinical implications***

Mechanisms of neoatherosclerosis and accelerated plaque formation after DES implantation are poorly elucidated and there is still a substantial lack of knowledge regarding the underlying mechanisms and causal factors. Recently, the role of lipid droplet-associated proteins such as those belonging to *Perilipin* family of proteins has been investigated in the context of atherogenesis. These proteins have a role in the excess accumulation of intracellular lipids and are linked to metabolic diseases, obesity, type 2 diabetes and

atherosclerosis.[40] Recently, the role of *adipophilin*, also known as *perilipin 2* or *adipose differentiation-related protein* (ADRP) has been implicated in the development of ISR due to neoatherosclerosis among patients implanted with 2nd generation DES. Of note, perilipin protein levels were significantly higher in peripheral blood mononuclear cells (PBMCs) of patients with in-stent neoatherosclerosis and implanted DES compared to patients with native CAD.[41]

Since rapid formation of lipid-laden neointima and extensive infiltration of foamy macrophages at the stent lesion site are histopathologic hallmarks of neoatherosclerosis, as previously elaborated, it is plausible that increased lipid retention and accumulation within resident macrophages and circulating monocytes that are being increasingly recruited to the lesion site are potential mechanisms involved in neoatherosclerosis formation and/or progression. Such effect might indeed be mediated by proteins, such as *perilipin 2*, that are involved in the regulation of cytoplasmic lipid droplets within macrophage foam cells and storage of cholesteryl esters derived from modified lipoproteins.[40, 42] Recently, experimental induction of *perilipin 2* deficiency and a concomitant increase in extracellular plasma cholesterol acceptors such as apolipoprotein A-I (apoA-I) and HDL cholesterol cumulatively reduced lipid droplets and cholesterol ester content in cultured macrophages and significantly reduced atheroma formation.[43]

***MicroRNA-based strategies in addressing complications of interventional treatment***

Current drug delivery solutions available with modern stents fail to selectively suppress proliferation of VSMCs without a negative impact on the growth of endothelial cells (ECs) within the vasculature. To overcome this issue, small non-coding RNA molecules that are able to distinctively regulate VSMCs and ECs might provide a viable solution. These molecules exert such effects through post-transcriptional silencing, degradation or

overexpression of genes and their downstream end-products that are involved in vascular remodeling.[44, 45] MicroRNAs could be utilized for the selective inhibition of VSMC proliferation, platelet activation and improvement of endothelial regeneration after stent deployment.[46] In fact, a selective miRNA-based strategy was able to markedly reduce restenosis, hypercoagulability and improve reendothelialization and vasodilatory response to acetylcholine in the preclinical model of vessel injury thus confirming the potential of this therapeutic approach in restricting or abolishing restenosis.[47, 48]

In the clinical practice, late stent failure due to neoatherosclerosis and stent thrombosis is a relevant problem because these events are associated with higher rates of fatal and non-fatal acute coronary syndromes (ACS) and of overall poor clinical outcomes.[49-51] Moreover, neoatherosclerosis is a frequent OCT finding in late or very late stent thrombosis.[14, 23, 52] Due to an increasing number of PCI procedures performed worldwide and currently unmet need in ISR prevention, it is expected that significant numbers of late stent failure events will occur in the future thus portending poor clinical outcome. Risk factors associated with the occurrence of neoatherosclerosis along with available therapeutic pathways that could mitigate neoatherosclerosis formation and/or progression are summarized in **Figure 3**.

## STENT-BASED STRATEGIES AND PROCEDULAR FACTORS IN THE CONTEXT OF NEOATHEROSCLEROSIS

During the last decade, DES implants have undergone substantial structural improvements, including thinner metallic struts and more biocompatible durable or biodegradable polymers thus often being labeled as “third-generation” DES. These modifications have led to an improved healing response and reduced failure potential

compared with first- and second-generation DES.[53] Stent design has a significant impact on flow dynamics in stented coronary segments. Thick struts affect local ESS, favoring the development of flow disturbances with low and oscillatory ESS, which may in turn activate the regenerating endothelium toward a pro-inflammatory phenotype and favor lipid uptake.[54] This may induce unfavorable healing response and development of neoatherosclerosis, although further studies are needed to confirm this hypothesis. On the other hand, thinner struts may improve re-endothelialization and reduce peri-strut inflammation and fibrin deposition.[55] In addition; the biodegradable polymer type and load may affect healing response and development of neoatherosclerosis, by modulating chronic intra-stent inflammation. However, there are no conclusive results on the relation between strut thickness and use of durable/biodegradable polymers with the formation of neoatherosclerosis. A recent OCT study by Guagliumi et al. showed a similar healing response after 3 months and incidence of NA after 18 months between a biodegradable polymer everolimus-eluting stent and a durable polymer zotarolimus-eluting stent.[56] In another study, the rate of in-stent neoatherosclerosis within biodegradable polymer biolimus-eluting stents was similar to bare metal stents, and tended to be lower than in durable polymer sirolimus-eluting stents.[57]

Furthermore, bioresorbable vascular scaffolds (BVS) were developed to only temporarily cover the diseased coronary segment followed by full biodegradation, potentially overcoming the long-term issues of metallic stents. However, randomized trials consistently observed worse long-term clinical outcomes of the Absorb BVS compared with metallic new-generation DES, both in terms of device-oriented adverse events and device thrombosis, particularly after 1 year. The international INVEST registry identified neoatherosclerosis as one of the main mechanisms underlying very late scaffold thrombosis, being observed in about 18% of lesions at 2-year follow-up.[58] More recently, Moriyama et al. conducted a study

with serial OCT imaging performed at baseline, 1 year, and 5 years after Absorb BVS implantation in 20 patients with 22 lesions. Neoatherosclerosis, defined as lipid-laden plaque including thin-cap fibroatheroma with or without intimal rupture and/or thrombi, and/or calcific plaque with or without neovascularization and/or macro- phage accumulation, was identified in 100% of lesions at 5 years after BVS implantation.[59] Multiple pathogenic factors have been suggested, including endothelial dysfunction, inflammation, and local blood flow alterations related to the scaffold strut size (i.e., thick struts), geometry, and large polymer load of BVS. Further studies are needed to clarify these potential mechanisms, and to identify the incidence of neoatherosclerosis after implantation of BVS other than the Absorb BVS.[60]

While the data corroborate that ABSORB BVS is a reasonably safe platform, it did not fulfill the promise of abolishing or reducing neoatherosclerosis and/or stent thrombosis. However, novel approaches in BVS polymer design that are under development might mitigate these late PCI complications. Of note, BVS with polymers based on poly-L-lactic acid (PLLA) might support beneficial vascular remodeling in humans without detected neoatherosclerosis at 2 years, even in the absence of antiproliferative drugs.[61] Previous preclinical studies on novel ultrahigh molecular weight amorphous PLLA BVS implants demonstrated expansive vascular remodeling in PLLA BVS and this biological phenomenon appeared independently of antiproliferative drugs.[62, 63] Furthermore, an addition of magnesium to BVS was associated with decreased thrombogenicity, less platelet adhesion and inflammatory cell deposition in porcine arteriovenous shunt model, compared to BMS.[64] In humans, a 12-month follow-up after implantation of the 2nd generation BVS with magnesium backbone showed a favorable safety profile and stable angiographic parameters between 6 and 12 months.[65] However, caution has to be exercised since De la Torre Hernandez et al recently demonstrated that the arterial healing process after implant deployment could be more device-specific rather than patient-specific, suggesting that not all

BVS scaffolds might act in the same manner in the long-term.[66] For this reason, future studies on newer generation magnesium- and PLLA-based scaffolds are, indeed, warranted to assess their effects on clinical endpoints during longer follow-up, particularly compared to established "workhorse" DES platforms.[67, 68]

Regarding the procedural PCI factors that might contribute to the formation of neoatherosclerosis, an OCT imaging provides accurate stent sizing and guidance of the stenting strategy, by providing both information on the lumen dimension and lesion characteristics.[69] Even more, latest ESC/EACTS guidelines on myocardial revascularization support the use of OCT imaging in cases dealing with an in-stent neoatherosclerosis and further procedure planning.[70] Post-PCI OCT imaging allows strut-level evaluation of the stent deployment and provides guidance for stent optimization. OCT is able to identify correctable abnormalities related to the stent and the underlying vessel wall, such as stent underexpansion, strut malapposition, and geographic plaque miss.[70] Stent underexpansion is a major predictor of early stent thrombosis or restenosis, however, no definite pathogenic link exists between acute strut malapposition and subsequent events.[70] In contrast, OCT studies investigating the mechanisms of stent thrombosis have consistently identified strut malapposition as a frequent underlying finding.[23] Whether stent optimization using an OCT imaging may impact on the development of neoatherosclerosis at follow-up remains unknown, and need to be demonstrated in future studies.

## PHARMACOTHERAPEUTIC STRATEGIES IN THE PREVENTION OF NEOATHEROSCLEROSIS

### *Role of antiproliferative drugs and the mode of drug delivery*

Sirolimus seems to reduce the amount of positive cell cycle regulators and increase the amount of cell cycle inhibitors, leading to inhibited cell migration, proliferation, and desensitization of the cells to the effects of low ESS.[1] Because durable polymers result in long-term drug sequestration within the polymer with prolonged drug delivery over time, this may result in long-term endothelial dysfunction. It seems plausible that limiting the duration of exposure of the arterial wall to mTOR inhibitors may result in a limited long-term endothelial dysfunction and perhaps decreased neoatherosclerosis though this is unproven to date.[71]

In the absence of definitive clinical data demonstrating differences between durable polymer DES (DP-DES) versus bioresorbable polymer DES (BP-DES) in terms of neoatherosclerosis, it is worth discussing what some of the advantages of the latter might be and why improvement in DES design is still necessary. As alluded to earlier, the genesis of late restenosis and neoatherosclerosis remains unknown but studies suggest it may be related to long-term effects on endothelium caused by -limus mTOR inhibitors used in the current generation DES. Long-term exposure of endothelial cells to sirolimus leads to endothelial barrier dysfunction allowing entrance of lipoproteins and immune cells into the arterial wall, similar to the pathogenesis of native vessel atherosclerosis.[72] Moreover, because drug tissue levels tend to decrease dramatically as the polymer degrades, the risk of neoatherosclerosis may actually be lower although more work is required to substantiate this claim. However, it remains unclear whether metallic surfaces are as biocompatible as polymer-coated surfaces (especially those coated with fluorinated polymers). While recent meta-analysis of randomized controlled trials showed that BP-DES are non-inferior to DP-DES, their potential advantages are yet to be demonstrated.[73]

***Antiplatelet and lipid-lowering therapy in the context of DES implantation***

Pharmacotherapeutic secondary prevention encompassing lipid reduction and inhibition of platelet activation, pathologic processes that are both implicated in neoatherosclerosis, is exceptionally important after DES implantation. The European Society of Cardiology (ESC) guidelines clearly emphasize the importance of these therapies after coronary revascularization.[74-76] The optimal duration of dual antiplatelet therapy (DAPT) with aspirin and a thienopyridine (ADP receptor/$P_2Y_{12}$ inhibitor) and/or addition of anticoagulants probably need to be calibrated on a personalized assessment of patients' coronary anatomy, comorbidities and ischemic/bleeding risk.[75, 77, 78]

Similarly, patients with ACS that underwent PCI are recommended to reach lower targets of low-density lipoprotein (LDL) cholesterol compared to conventional patients with hyperlipidemia.[79] However, these goals are often unmet in population of ACS patients due to statin intolerance or refractoriness to LDL cholesterol-lowering despite optimal high-potency statin therapy or statin combined with the intestinal cholesterol inhibitor ezetimibe. Due to residual cholesterol and inflammatory risk in these patients, therapies that would provide additional reduction in circulating levels of LDL cholesterol and proinflammatory mediators and would contribute to a greater plaque stabilization are required.[80] Recently, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors were shown to increase recycling of LDL cholesterol receptors, therefore, efficiently lowering concentrations of LDL cholesterol in circulation beyond the effects of standard therapy.[81] In the context of CAD, GLAGOV trial showed that PCSK9 inhibition on top of statin treatment was associated with a significant additional reduction of plaque burden in patients with CAD, compared to patients that were using statins only.[82] This aggressive lipid-lowering treatment with PCSK9 inhibitors might have an impact on lipid-related neoatherosclerosis and plaque stabilization since higher levels of matured PCSK9 were associated with coronary spotty calcifications in ACS patients and this concept was also confirmed in a preclinical study by Goettsch and colleagues.[83, 84]

Finally, precision-tuning of the lipid-lowering therapies might be based on the plaque imaging (i.e., assessment of fibrous cap thickness in vulnerable plaque lesions, quantification of plaque burden) and geometric characterization of the vessel architecture at lesions of interest (i.e., minimal luminal area, etc.) in each individual patient as advanced imaging techniques become more routinely used in the clinical practice.[85]

***IL-1/IL-6/CRP pathway***

The inflammation is an important driver of healing responses after stent implantation.[86] Therefore, targeting proinflammatory pathways while exerting immunosuppressive effects seems as a reasonable therapeutic approach to prevent in-stent restenosis and late complications of interventional treatment.[87] The pivotal CANTOS trial demonstrated that inflammatory pathogenesis of atherosclerosis is a validated concept showing that the inhibition of *interleukin-1 beta* (IL-1β) with a therapeutic monoclonal antibody *canakinumab*, without affecting lipid levels, in patients with previous acute myocardial infarction (AMI) and CRP levels >2 mg/L was able to significantly lower the composite outcome of MI, stroke or death by 15% and secondary endpoint that included urgent revascularization by 17%, compared to placebo.[88] This notion was confirmed in a study by Chibana et al. showing that, in patients implanted with the mTOR-inhibitor-eluting stent, *sirolimus* increased IL-1β mRNA expression and increased IL-1β release within coronary artery smooth muscle cells (CASMCs) implicating that serum IL-1β levels could serve as a biomarker for DES-associated coronary endothelial dysfunction.[89] Perhaps such effects of inflammation-directed pharmacotherapy that inhibits IL-1β pathway could extend to the suppression of neoatherosclerosis due to the implicated role of this cellular pathway in foam cell formation and induction of interleukin-6 (IL-6) in human endothelial cells.[90] Anti-inflammatory agents might indeed ameliorate residual risk among patients that survived AMI since standard

pharmacotherapy including high-dose statin regimen is often insufficient in preventing recurrent ischemic events or death.[91]

The pharmacologic blockade of other mediators of inflammation such as *interleukin-6 receptors* (IL-6R), *CC2 chemokine receptors* and *CD20* might also prove as viable strategies in the future therapeutic targeting of vascular inflammation and atherothrombosis prevention.[92]

So-called *"C-reactive protein (CRP)/IL-6/IL-1 axis"* recently became a research hotspot in the anti-inflammation secondary prevention pharmacotherapy with agents such as low-dose methotrexate and low-dose colchicine being currently investigated in clinical trials for the purpose of reducing cardiovascular events.[93-95] A role of NLRP3 inflammasome that is activated by cholesterol crystals and implicated in atherosclerosis has been increasingly studied with a potential of well-known "old" agents such as colchicine finding a new role in cardiovascular applications.[96] A recent study by Vaidya et al. showed that the addition of low dose colchicine (0.5 mg/day), which inhibits cholesterol crystal-induced activation of inflammasome, to optimal medical therapy was associated to a significant reduction of plaque volume at coronary computerized-tomography (CCT) and of hs-CRP levels among patients with recent ACS (<1 month), compared to optimal medical treatment only.[97] This study showed that colchicine might be a valuable addition to secondary prevention armamentarium in patients that suffered ACS and because of its anti-inflammatory properties, it is plausible that colchicine could mitigate some of the complications of DES implantation. However, to establish whether these beneficial effects of colchicine on surrogate end-points translate into a benefit on clinical outcomes remains to be investigated.[98]

***NF – kappa B pathway***

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway is an intracellular signal transduction system that is implicated in multiple inflammatory disorders, cancer proliferation, innate immunity, cellular apoptosis, and differentiation.[99] It is a *"rapid-acting"* and pleiotropic transcription factor meaning that it is ubiquitously present in cells in an inactive form and does not require a *de novo* protein synthesis for its activation. Activation mechanisms of the NF-κB pathway are multiple and include the canonical, non-canonical and the atypical activation cascades.[100] Canonical pathway of NF-κB activation is mediated by Toll-like receptors (TLRs), interleukin-1 receptors (IL-1Rs), tumor necrosis factor receptors (TNFRs) and various antigen receptors such as lipopolysaccharides (LPS) from the bacterial cell wall and viral antigens.[101] Activation of NF-κB dominantly occurs via IkappaB kinase (IKK) complex-mediated phosphorylation of inhibitory molecules such as IkappaBalpha that seems to play a central role in the initial convergence of most stimuli that can activate NF-κB signal transduction.[102-104] In the context of inflammatory effects, NF-κB pathway regulates proinflammatory cytokine production, leukocyte recruitment, and cell survival thus contributing to cellular inflammatory response.[105, 107]

Previously, a link between NF-κB pathway and atherosclerosis has been established, involving all stages of atheroma development, starting with plaque formation and subsequent destabilization and rupture.[107, 108] Additionally, NF-κB pathway activation is involved in lipid metabolism, foam cell formation, vascular inflammation, proliferation of VSMCs, arterial calcification, cardiac fibrosis, and plaque progression making it as a worthwhile therapeutic target in cardiovascular disease.[109-112] In the preclinical model, inhibition of NF-κB pathway abolished induction of adhesion molecules in endothelial cells, impaired macrophage recruitment to atherosclerotic lesions and significantly decreased expression of proinflammatory cytokines and chemokines in aorta thus clearly demonstrating that NF-κB signaling is an important cross-talk mediator of atherosclerosis and its therapeutic blockade

could portend myocardial protection.[113] In this respect, the use methotrexate, an immunosuppressive and antimetabolic agent that inhibits T-cell activation, intracellular adhesion molecule expression, folate metabolism and methyltransferase activity and selectively downregulates B-cells expression has been recently examined in terms of potential cardiovascular pharmacologic applications.[114, 115] Importantly, methotrexate blocks the binding of interleukin-1β (IL-1β) to interleukin-1 receptors (IL-1Rs) and inhibits production of cytokines such as interleukin-4 (IL-4), interleukin-13 (IL-13), interferon gamma (IFN-γ) and TNF-α.[116, 117] Finally, methotrexate suppresses TNF-induced NF-κB pathway activation by inhibition of NF-κB-dependent reporter gene expression.[118] Another mechanism by which methotrexate decreases basal levels of NF-κB activity is through increased expression of long intergenic (noncoding) RNA-p21 levels via DNA-dependent protein kinase catalytic subunit (DNA PKcs)-dependent mechanism.[119] Furthermore, adenosine selectively suppressed TNF-induced NF-κB activation in different cell types.[120] Mechanisms of methotrexate interaction with NF-κB pathway are illustrated in **Figure 4.**

Stent-based methotrexate delivery in a porcine coronary artery was able to effectively attenuate peristrut inflammation and neointimal hyperplasia.[121] Favorable effects of methotrexate on the formation of neoatherosclerosis in a rabbit model of atherosclerosis with implanted DES were recently reported.[122] In humans, a recent clinical study that involved patients with elective PCI showed that low dose (5 mg/week) of oral methotrexate administration before and after the procedure was safe and no cases of clinical restenosis were reported at 9-month follow-up, however, BMSs were used in this setting.[123]

Finally, it seems that therapies such as colchicine and methotrexate have a potential of lowering cardiovascular risk through immunomodulation.[124] If these effects might extend to complications of the coronary interventional treatment such as neoatherosclerosis remains to be determined.

**Soluble TREM-1 as a marker of in-stent restenosis and potential pharmacologic target**

Triggering receptor expressed on myeloid cells (TREM)-1 is the transmembrane glycoprotein receptor of the immunoglobulin superfamily, involved in activation of monocytes/macrophages and neutrophils by signaling through adapter protein DAP12.[125] This protein is implicated in the critical regulation of acute inflammatory responses by amplifying Toll-like receptor (TLR)-initiated innate immune responses against microbes and by upregulating proinflammatory chemokines and cytokines in response to fungal and bacterial antigens.[126] Several studies showed that cellular interference with TREM-1 pathway conferred a protective effect in the setting of inflammatory bowel disease, septic shock and ischemic myocardial injury.[127-129] In a study by Boufenzer et al. the soluble form of TREM-1 (sTREM-1) detected in plasma was a reliable marker of TREM-1 activation and was detectable in AMI patients with its concentration level being the independent predictor of death.[129] In recent times TREM-1 has been marked as one of the orchestrators of the inflammatory response that ensues after AMI since TREM-1 deletion or experimental modulation by short inhibitory peptide reduced myocardial inflammation, attenuated leukocyte recruitment and finally, improved overall heart function and survival in animal models.[130] During and after the AMI, necrotic cellular fragments and extracellular matrix components that are released in the bloodstream produce many damage-associated molecular patterns (DAMPS) that, in turn, activate pattern recognition receptors (PRR). In this cascade of events, TREM-1 plays an important role since it interacts with multiple PRRs, especially with TLR2 and TLR4, receptors with the highest expression levels in the myocardium.[126, 131]

Due to these established implications of TREM-1 signaling in cardiovascular pathology, a study by Wang et al. enrolled 130 patients with angiography-determined ISR and age- and gender-matched control group of 150 patients without ISR that were finally selected among the pool of 1683 patients that underwent PCI.[132] This study showed that sTREM-1 levels in

serum were significantly increased in patients with angiography-determined ISR when compared to controls. Furthermore, blockade of TREM-1 with a synthetic inhibitory peptide LP17 significantly inhibited while TREM-1-activating antibody promoted increased proliferation, migration of VSMCs and cellular inflammation. This study told us two important things – sTREM-1 levels in serum might reflect the presence and degree of ISR among patients that underwent stent implantation thus serving as a predictive biomarker in this setting and on the other hand, pharmacologic inhibition of TREM-1 might help in attenuating restenosis progression. Interestingly, TREM-1 expression in macrophages is regulated at transcriptional level by NF-kB pathway that was previously elaborated in this review and experimental data showed that cellular treatment with inhibitors of NF-kB was able to abolish the expression of message of TREM-1 induced by bacterial stimuli.[133] Mechanism of TREM-1 pathway and its interaction with NF-kB pathway are depicted in **Figure 5.**

Taken together, preclinical, and clinical data on TREM-1 warrant further investigations of this peptide in the ACS setting and in the risk management/monitoring for complications after stent implantation.

***Future perspectives***

It becomes evident that research directed towards novel stent systems and adjunct pharmacologic solutions will mark the upcoming era of cardiovascular science, as we delve deeper in the pathophysiology and vascular biology of coronary artery disease and both local and systemic immuno-inflammatory responses that are associated with the nature and complications of interventional treatment. In this regard, the systemic administration of low-dose immunosuppressive and anti-inflammatory pharmaceutical agents seems as an attractive concept that showed some promise in modifying the course and complications of

atherosclerotic disease. How these effects will translate to patient bed and real-life clinical outcomes remain to be seen in the upcoming trials, especially those conducted in the secondary prevention setting.

## CONCLUSIONS

While tremendous improvements have been made in minimizing the late complications of coronary interventional treatment, risks of neoatheroslerosis and late stent failure are still present, requiring the skillful precision tailoring of antiplatelet and antiinflammation pharmacotherapy combined with the appropriate stent choice placed in the correct vessel with correct apposition, size, and expansion in the correctly-selected patient. Local delivery of the pharmaceutical agent to the site of the lesion that will secure long-term patency with preserved endothelial function remains to be the quest for the interventionalist's Holy Grail. This quest will remain even more complicated with challenges in reducing hyperlipidemia, suppressing inflammation, slowing physiological drivers of thrombosis and, finally, recognizing the residual cardiovascular risk among patients with a significant coronary artery disease and high comorbidity burden that underwent PCI.

**Supplementary material:** References 51 through 124 are available in the **Appendix A**.

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## FIGURE LEGENDS

**Figure 1.** Graph depicting the percentage of atherosclerotic change in drug-eluting stents (DES) vs. bare-metal stents (BMS) in relation to implant duration in months (based on the autopsy studies). Reproduced based on the *Nakazawa et al. JACC: Cardiovascular Imaging, 2009;2:625-628.*

**Figure 2.** Basic pathogenic mechanisms of native atherosclerosis and in-stent neoatherosclerosis. **A-B.** Lipid diffusion and inflammatory cell migration through the endothelium is similar between native atherosclerosis and in-stent neoatherosclerosis but potentially accelerated in the latter due to the presence of an insufficiently regenerated endothelium and the effects of drug-induced inhibition of re-endothelialization processes. The underlying atherosclerotic plaque might contribute to the growth of neointima both with expansion through the stent struts and with the release of proinflammatory factors. Furthermore, chronic foreign body reaction to the stent may exacerbate these mechanisms thus accelerating neoatherosclerosis progression (red arrows), **C-D**. Representative intracoronary optical coherence tomography (OCT) images showing a native lipid plaque within the vessel **(C)** and a neoatherosclerotic lesion characterized by the lipid-laden neointima **(D)**.

**Figure 3.** A summary of risk factors associated with neoatherosclerosis and currently available pharmacotherapeutic agents that might address pathophysiologic pathways associated with the formation and progression of neoatherosclerosis after stent implantation due to an acute coronary syndrome. For the making of this figure, illustration elements that were kindly provided by the Servier were used. Servier Medical Art is licensed under a Creative Commons Attribution V.3.0 Unported License.

**Figure 4.** A scheme showing the effects of methotrexate on NF-kB pathway and pleiotropic roles of NF-kB pathway in the pathophysiology of the disease.

**Figure 5.** A scheme showing the effects of TREM-1 pathway activation and its implicated role in in-stent restenosis and cellular inflammation. TREM-1 blockade by an experimental pharmacologic agent is also presented.

**Table 1.** Independent clinical risk factors that are associated with late or very late in-stent restenosis due to neoatherosclerosis

| **Late or very late neoatherosclerosis (>12 months or >36 months)** | |
|---|---|
| **Risk factors** | **PMID** |
| Presence of peristrut microhemorrhages | 27865168 |
| Increased out-stent plaque volume (OSPVI) | 29066157 |
| Increased mean neointimal thickness (NIT) | 23891431 |
| Stent length | 28943494 |
| Stent fracture | 29151488 |
| „Off-label“ DES usage | 23227328 |
| Localized hypersensitivity reaction | 25423451 |
| DES as an used stent type | 22798521, 29066157 |
| Duration of DES implant >48 months, increased stent age | 25613674, 22798521 |
| DES duration > 20 months | 21646494 |
| BVS duration of 5 years or more | 29699614 |
| Current smoking | 22798521 |
| Chronic kidney disease | 25613674, 22798521 |
| Diabetes mellitus and HbA1c levels >7%,<br>Insulin resistance | 24514877, 26413014 |
| >70 mg/dL of LDL cholesterol at OCT follow-up | 25613674 |
| Absence of ACE-Is or ARBs in post-discharge pharmacotherapy | 22798521 |
| **Early neoatherosclerosis (<12 months)** | |
| Arterial hypertension and high pre-stent LDL cholesterol levels | 26385044 |

**Abbreviations: ACE Is**-angiotensin-converting enzyme inhibitors; **ARBs**-angiotensin 2 receptor blockers; **BVS**-bioresorbable vascular scaffold; **DES**-drug-eluting stent; **LDL**-low-density lipoprotein; **PMID**-PubMed ID number

Figure 1.

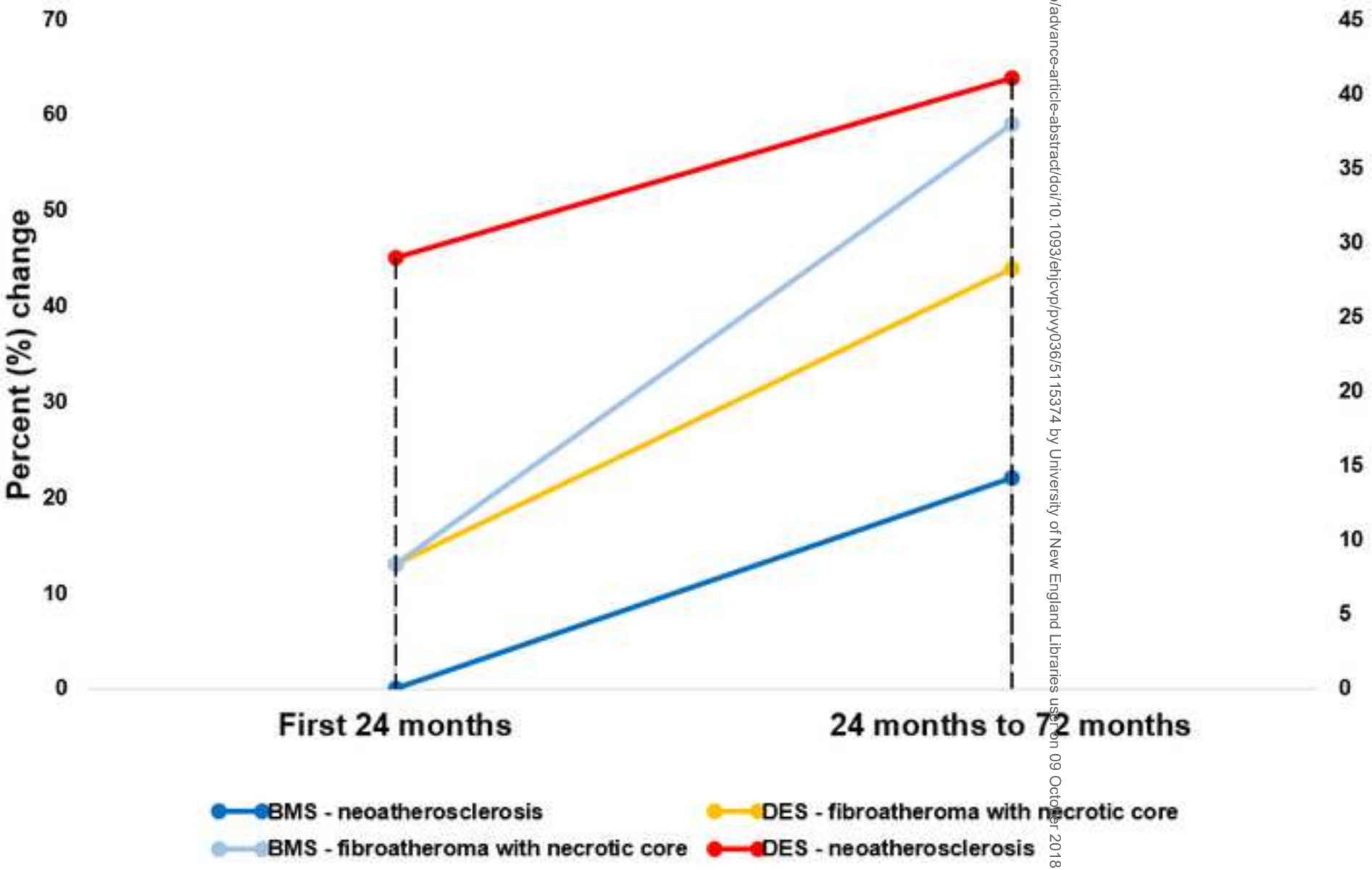

Figure 2.

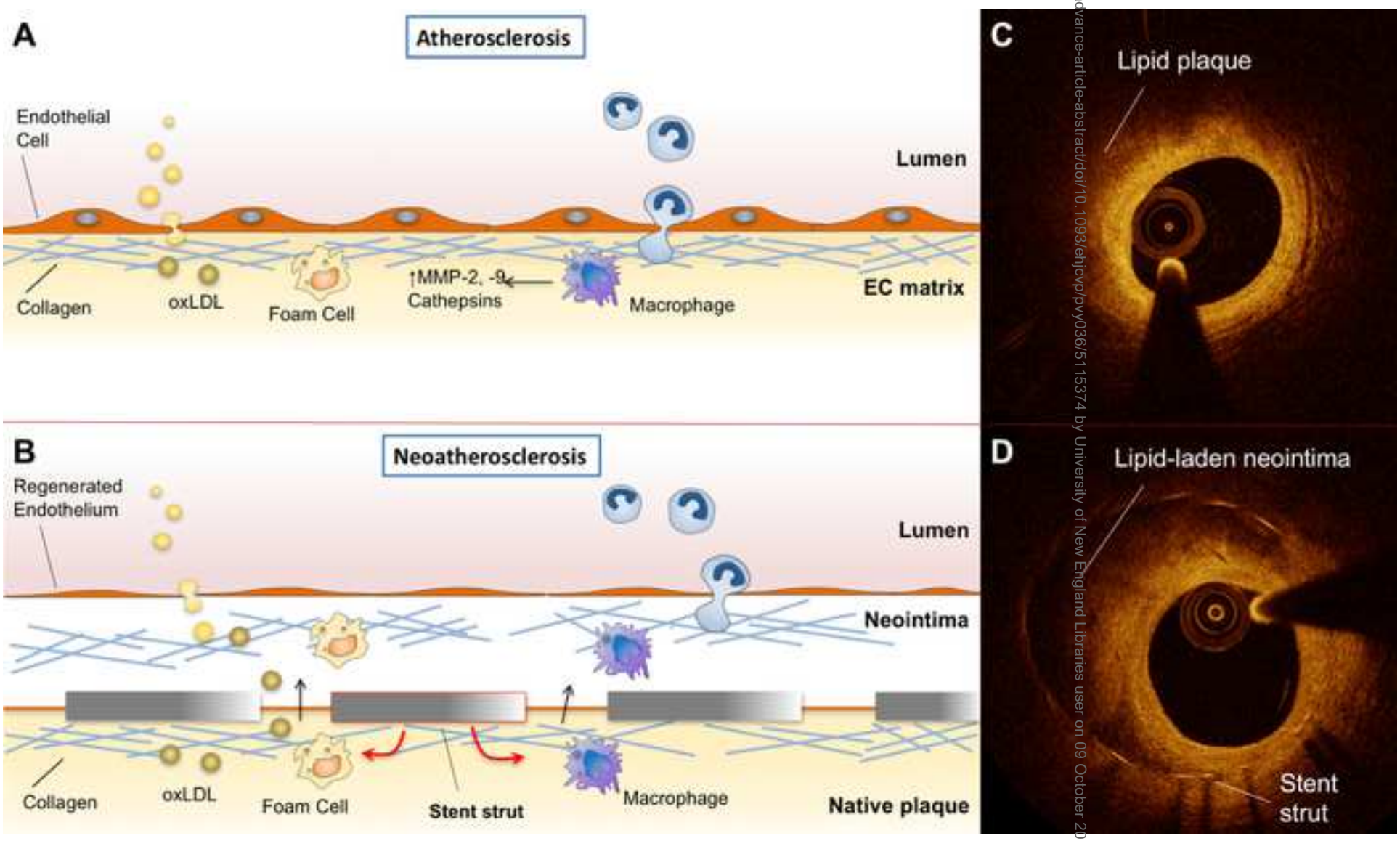

Figure 3.

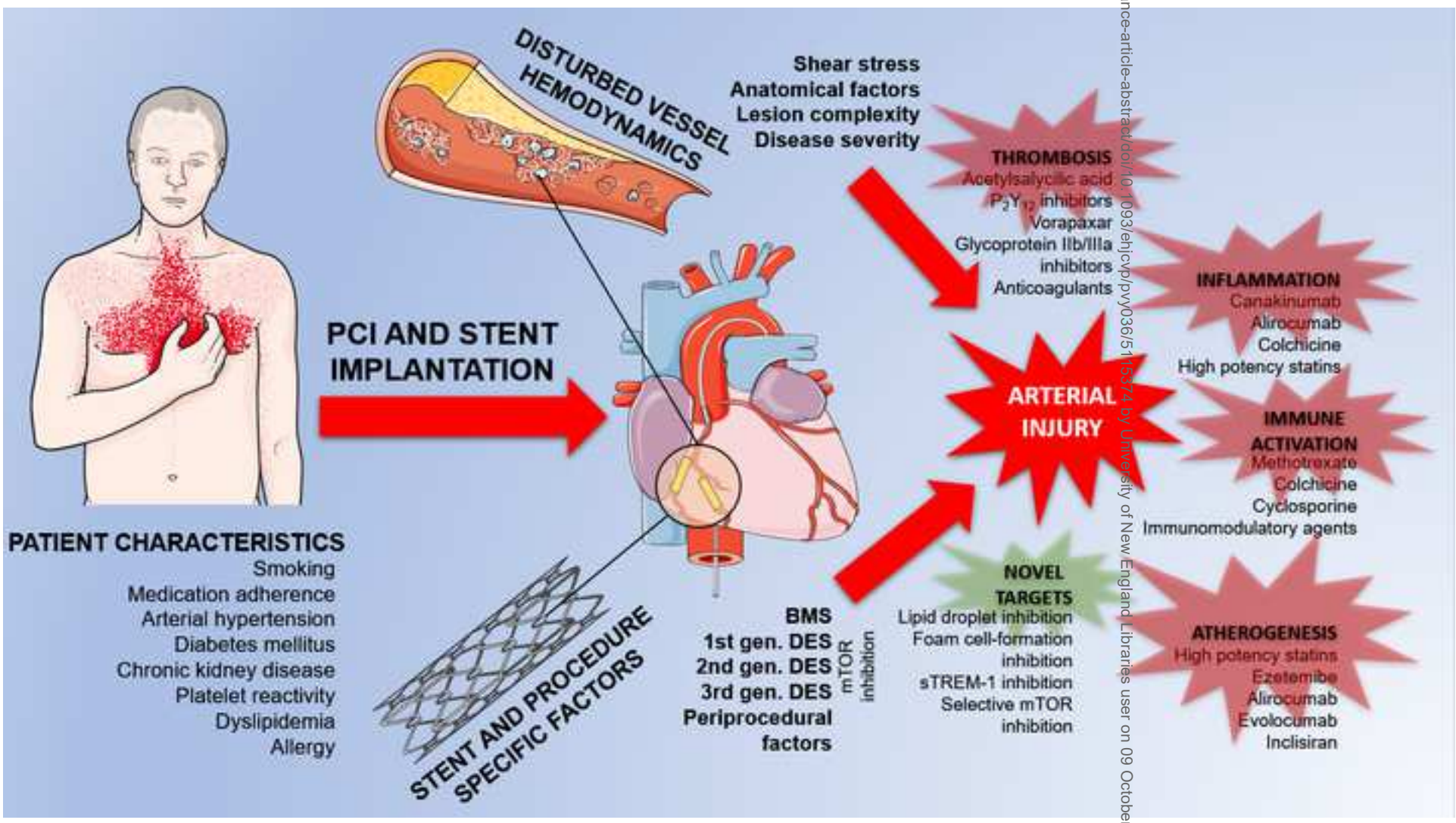

Figure 4.

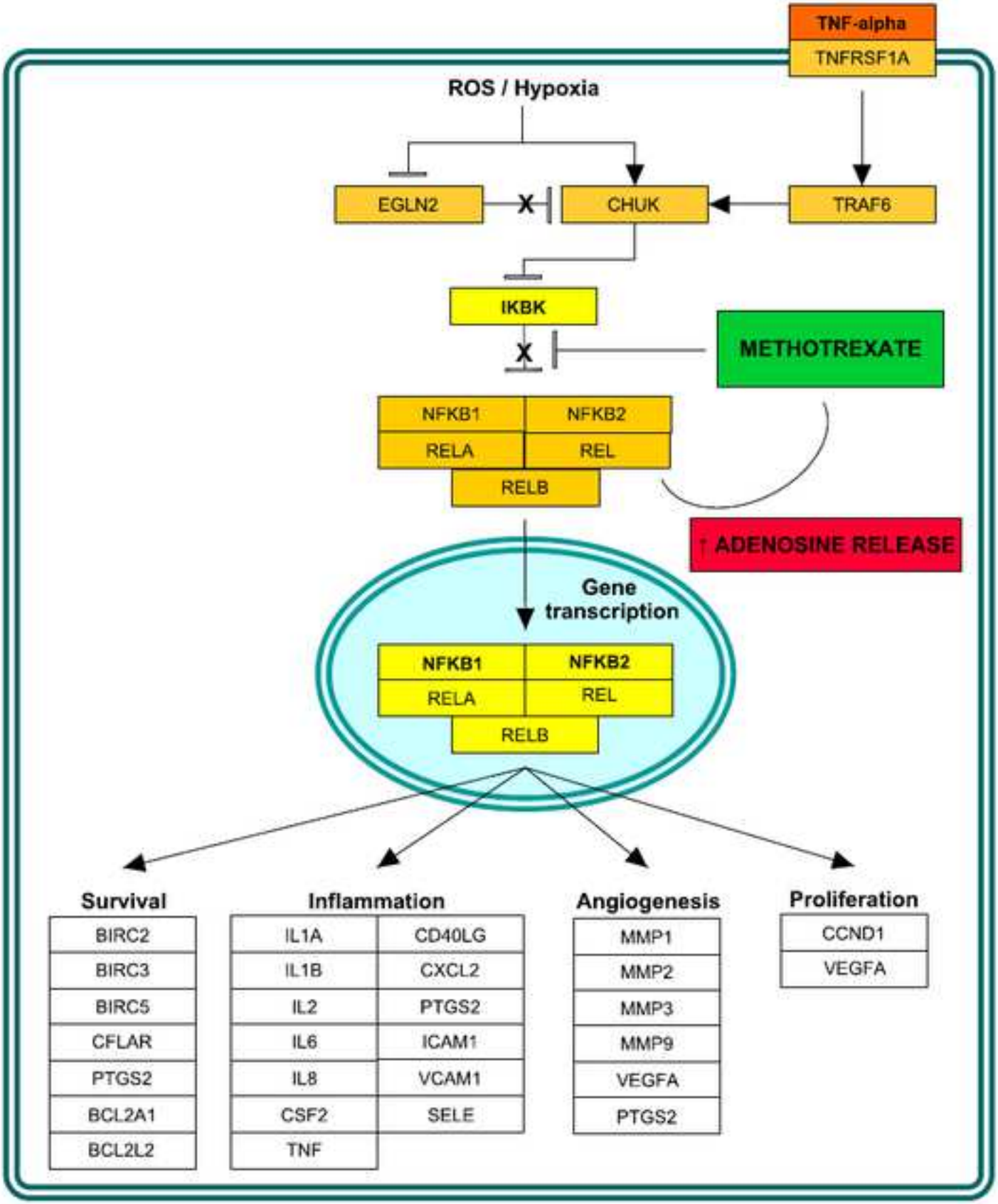

Figure 5.