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# Adeno-Associated Virus-Based Transgene Delivery for Treating Progressive Vascular Diseases

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**Abstract**

There has been much progress in the clinical treatment of Progressive Vascular Diseases (PVD) since the introduction of statins, calcium channel blockers, and other relevant drugs. Yet long term PVD still plagues much of our aged population. The use of gene therapy, delivering therapeutic transgenes, should provide both long-term and safe treatment, as well as more physiologically relevant treatments. Adeno-associated Virus (AAV) can provide long term and safe gene delivery, very appropriate for long term PVD. In regard to therapeutic transgenes for the treatment of PVD, there is a choice of several gene categories, all of which may provide a significant level of efficacy yet have different metabolic modes of action. As examples there are anti-inflammatory cytokine genes, blood lipoprotein-metabolism genes, and anti-oxidant genes.

## Introduction

The various forms of PVD, most notably atherosclerosis, encompass a large percentage of the morbidity and mortality of the aged population, and challenges cancer for the highest position of mortality [1]. As opposed to small molecule drugs (pills), the uses of genes (and their encoded proteins) which act in regulating the disease process should provide more physiologically relevant treatments with higher efficacy. Atherosclerotic plaque, particularly in its intermediate, most common form, can be thought of as an immune cell tumor. Almost all types of immune cells have been found within atherosclerotic plaque and have been deemed as causative agents. Monocytes, macrophages and derived foam cells are present in abundance within developing atherosclerotic plaques, however it remains to be proven which of these immune cell types are the mechanistic driver, the etiologic agents, of atherogenesis and PVD [2,3]. For example T cells have also been found to be elevated in atherosclerotic plaque, and these cells have also been suggested as critical mechanistic drivers of PVD [4].

## The Viral Vectors Available

There are three main virus types used for transgene delivery (gene therapy). These are adenoviruses (Ad) [5], retrovirus/lentivirus [6] (usually Human Immunodeficiency Virus-Based, HIV), and Adeno-associated Virus (AAV) [7]. Figure 1, shows the use of these three types over time for the treatment of PVD. It can be seen in Figure 1A that the use of Ad is on decline over time, and this is likely due to its short term expression, its association with inflammation, and its association with adverse reactions (death, inflammatory reactions) during its use (all negatives for the treatment of PVD. The use of lentiviral (retrovirus) vectors, in Figure 1B, are on the strong upswing, yet retroviruses as a general type are also known to be linked to adverse reactions and often with truncated expression periods. The worst documented retrovirus adverse reaction is the development of cancer, in both animal models and in patients. AAV is the focus here as it gives both long term transgene delivery (needed for the treatment of long-term vascular diseases) and its high level of safety.

Regarding AAV, since its first use in 1984 [7,8] for gene delivery AAV is a vector of continuous growing popularity (Figure 1C), and for good reason. AAV gene delivery has been shown to last at least ten years in patients [9] and has had no adverse reactions attributed to it in clinical trials. Moreover, AAV is the only gene therapy vector which has been approved as standard of care use in humans (in Europe) [10]. There are over 100 AAV types known and now many new synthetically altered capsid types are being generated every year [11]. The liver appears to be a major reservoir for infection (gene delivery) by many AAV types, as are various types of muscle. In arteries the arterial smooth muscle cells are the primary target for AAV type 2, as well as many other AAV types [12].

## The Therapeutic Transgene Approaches

There are a number of gene therapy approaches which might be taken to prevent or slow down atherosclerosis. The three that will be covered here include transgenes which down-regulate the immune system, dyslipidemia in the circulation and arterial wall or which inhibit reactive oxygen species.

## Down-Regulation of the Immune System/Anti-Inflammation

As it is unclear which immune cell type, and which specific type of inflammation, is at the heart of atherogenesis the use of a general anti-inflammatory genes would seem to be advantageous for anti-atherosclerosis effect [13,14]. The two most well known, well studied genes include transforming growth factor beta 1 (TGFβ1) and interleukin 10 (IL10). TGFβ1 is very pleiomorphic in its effects, yet its broad negative effects on the immune system are well documented [15]. Perhaps its most prominent adverse effect is its association with the induction of fibrosis [16]. Thus perhaps at this time TGFβ1 is not a desirable transgene for treating vascular disease due to its complicated and divergent effects.

IL10 [17] may be superior to TGFβ1 as fewer complications have been attributed to it, yet it has also been associated with it a number of adverse reactions such as anemia, and increased infections [18]. Regarding desirable therapeutic effect IL-10 has a broad effect on

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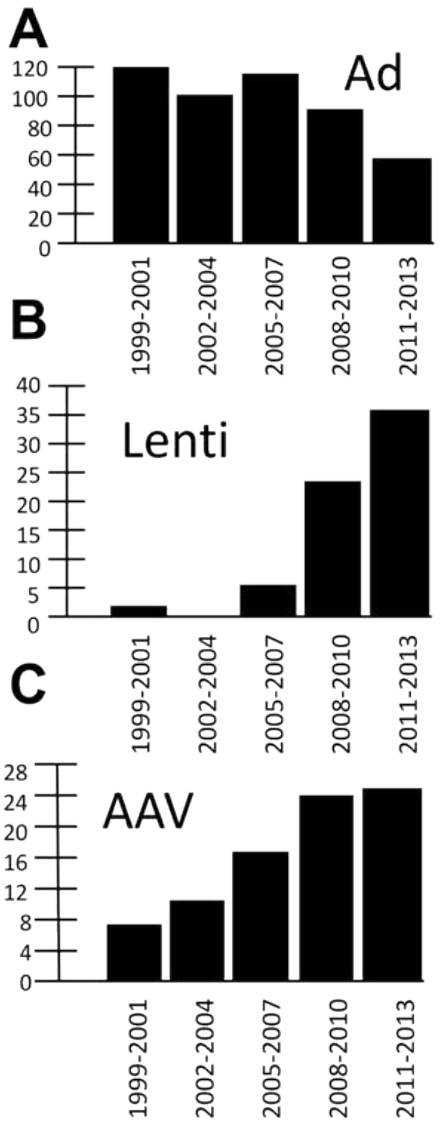


**Figure 1:** Use of viral vectors for treating PVD measured by publications. Ovid/medline search provided these publication numbers. PVD manuscripts were compiled by combined searching of "atherosclerosis" or "atherogenesis" or "cardiovascular" or "ischemia reperfusion" or "restenosis" or "hypertension". The compiled PVD manuscripts were then crossed with, and, searches of a particular viral vector and the indicated set of years.

multiple immune cell types. In particular, IL-10 decreases Mo/Mac activation, recruitment and proliferation, which are likely involved in plaque development [19]. Moreover, and importantly, the IL10 transgene has been studied by multiple groups using AAV delivery, and all have shown it to have significant efficacy in animal models [20-24]. Like most cytokines, IL10 has a very short half-life in blood and because of this we consider that gene therapy is the most advantageous way to use IL10 therapeutically. This is because the gene has a much longer half-life than the protein. Again, AAV has the longest documented transgene life in patients [9].

Both IL-10 and TGFβ1 are Th1 response cytokines and there are other cytokines which are in this category which may be useful for inhibiting immune response within arteries. As cytokines are secreted proteins and largely act through diffusion and blood transport both the arterial wall and the liver are both appropriate platform tissues to target gene therapy, with the arterial wall, itself, being the most desirable approach.

### Down-regulation of dyslipidemia

The level and makeup of lipoproteins in the blood are critical in directing vascular clinical outcome towards atherosclerosis or not [25]. High Density Lipoprotein (HDL), a complex assembly of proteins and lipids, gives desirable trafficking of lipids/lipoproteins which results in lower lipid levels (e.g. cholesterol) out of arterial walls. Apo A1 is one of the major protein components of HDL, thus higher Apo A1 levels would be one potential strategy for regulating dylipidemia [26,27]. A number of studies have shown that Apo A1 gene delivery offers additional protection against PVD, particularly atherosclerosis. Additionally, there is one natural mutation to the ApoA1 gene, Milano, which appears to have higher athero-protective attributes than wild type [28]. This desirable mutant gene has also been used therapeutically by gene delivery and has shown efficacy against PVD/atherosclerosis [29].

Conversely, Low Density Lipoprotein (LDL) is undesirable at high levels, is associated with atherogenesis, and at high levels would be considered a dyslipidemia. ApoB100 is a major protein component of LDL. Thus lowering ApoB100 might be an approach towards lowering vascular disease. The use of shRNA genes, or other inhibitory gene-type agents inhibiting ApoB100 expression, might be an additional strategy to limit PVD/atherosclerosis [30].

### Down-regulation of reactive oxygen species

When are present in all cells and are eliminated by antioxidants. When Reactive Oxygen Species (ROS) are produced at high levels, above what the normal endogenous antioxidant defenses can handle, then oxidative stress takes place. Of course ROS are very important because of the widespread damage it can cause through its alteration of cellular proteins, DNA, and lipids. Moreover, ROS is a factor in many diseases of aging, and its role in cardiovascular disease is well documented [31]. It is clear that ROS intersects with and promotes both inflammation and dyslipidemia in their roles in causing atherogenesis.

As ROS is an ever present problem cells encode a number of anti-oxidant genes. These include superoxide dismutases (SODs), glutathione peroxidases (GPXs), catalases, glutaredoxins. thioredoxin peroxidases (peroxiredoxins, eg. PRDX6), and others [32]. We have recently shown that AAV-delivered PRDX6 is able to inhibit atherosclerosis in Low Density Lipoprotein Receptor Knockout mice (LDLR KO) on high cholesterol diet [33].

## Summary

In summary the future is promising for the development of AAV-based gene therapies for limiting PVD, with many possible approaches likely to show significant efficacy.

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