Beyond Anti-VEGFs – Anti-Insulin Receptor Substrate-1 Oligonucleotides as a Novel Approach to Ocular Neovascular Disorders

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Abstract

Angiogenesis is a complex process that is vital to health but is also a driving factor behind a broad range of malignant, ischaemic, inflammatory, infectious and immune disorders. For optimal efficacy and safety, therapies aimed at preventing angiogenic-mediated disorders must differentiate between healthy and pathological angiogenesis or neovascularisation. Aganirsen is an antisense oligonucleotide that inhibits the insulin receptor substrate (IRS)-1 angiogenic pathway by targeting the IRS-1 messenger RNA. To date, studies of aganirsen have focused mainly on ocular disorders because of the ability to assess non-invasively the effect of the drug on neovascularisation and to address the unmet need for effective therapies in these blinding disorders. Aganirsen (GS-101) eye drops inhibit progressive corneal neovascularisation and appear to be well tolerated. The drug may offer an alternative and/or adjunct to intraocular anti-vascular endothelial cell growth factor (VEGF) agents, which are the current reference standards to prevent neovascularisation in retinal diseases. This is because it has a different and potentially complementary mechanism of action and can be administered topically. Antisense oligonucleotides targeting IRS-1 may present a valuable new approach to control pathological angiogenesis in the eye and elsewhere.

Keywords

Aganirsen (GS-101), antisense oligonucleotide, insulin receptor substrate-1, ocular disorders, topical anti-angiogenic therapy, adjunct to anti-VEGF, healthy and pathological angiogenesis

Disclosure: James W Bainbridge is a NIHR Research Professor and is supported by the NIHR Biomedical Research Centre for Ophthalmology at Moorfields and UCL. He has participated in one clinical trial workshop sponsored by Gene Signal. Claus Cursiefen and Vanya Loroch are consultants to Gene Signal. Eric Viaud is the Chief Executive Officer of Gene Signal International SA.

Received: 15 June 2012 **Accepted:** 23 July 2012 **Citation:** *European Ophthalmic Review*, 2012;6(3):190–3 *DOI: 10.17925/EOR.2012.06.03.190*

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Support: The publication of this article was funded by Gene Signal.

Angiogenesis and its Importance in Health and Disease

The formation of new blood vessels, commonly called 'angiogenesis', comprises several processes that include vasculogenesis, where stem cells or angioblasts differentiate into blood vessels and which occurs in developing, initially avascular embryos and to a certain extent in adults;[1] and angiogenesis *per se*, where new blood vessels (especially capillaries) grow from pre-existing vessels by sprouting or branching[2] and which is the prevailing mechanism in adults.[3]

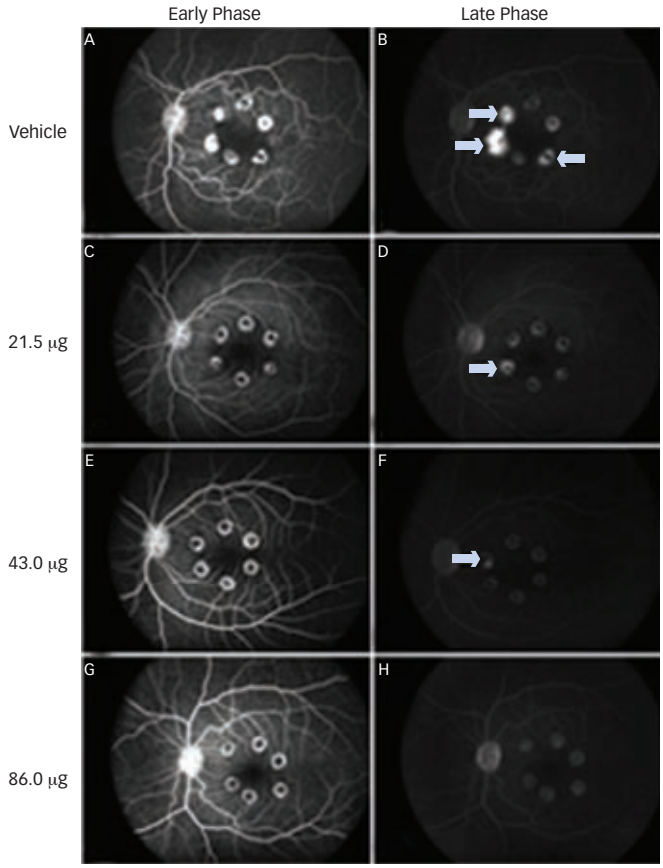
Angiogenesis is necessary to supply tissues with nutrients and oxygen, and is an integral mechanism in many physiological processes, such as wound healing and tissue growth and repair,[4] muscle development[5] and ovulation.[6] At the same time, disruption of the fine balance between factors that induce blood vessel formation and those that inhibit or halt the process can result in pathological angiogenesis, or neovascularisation, leading to increased formation of blood vessels that may be excessive or occur in normally avascular tissue. For example, rapid and persistent growth of new blood vessels is a hallmark of cancer, while excessive angiogenesis can provide a route of entry for inflammatory cells into sites of chronic injury (e.g. Crohn's disease), contributes to the increased epithelial cell turnover and skin plaques in psoriasis and causes ocular diseases that can lead to blindess.[7] Neovascularisation is implicated in a multitude of malignant, ischaemic, inflammatory, infectious and immune disorders.[3]

As angiogenesis is so vital for health, any therapeutic strategy aimed at treating angiogenic-mediated disease must discriminate between physiological and pathological angiogenesis to ensure that only neovascularisation is affected. It is also important to recognise that all angiogenic mechanisms may participate in neovascularisation to various extents in different diseases. A thorough understanding of the processes controlling angiogenesis and gaining access to as many molecular targets in the relevant regulatory pathways as possible is essential in developing an appropriate therapy.

Neovascularisation in Ocular Diseases

Studying neovascularisation *in vivo* is greatly facilitated if blood vessel development can be assessed non-invasively. As a result of the unique optical transparency of the ocular media, angiogenic diseases

Figure 1: Aganirsen Eye Drops Significantly Reduce the Extent of Experimental Choroidal Neovascularisation Lesions in Non-human Primate Eyes



Representative early (30-second) and late-phase (6-minute) fluorescein angiograms collected four weeks after laser photocoagulation from vehicle-treated animals (A and B) and from those receiving twice-daily doses of Aganirsen 21.5 µg (C and D), 43 µg (E and F), or 86 µg (G and H) from two days before laser photocoagulation to 14 days after it. Arrows indicate grade IV CNV lesions. Source: Cloutier, et al., 2012.[30]

of the eye offer a unique opportunity to study neovascularisation and the impact of intervention in the living organ.

Ocular neovascularisation can lead to irreversible visual impairment, whether by causing permanent harmful changes to the neuronal architecture of the retina or by making the cornea opaque.[8,9] There is a universally recognised need for effective therapies in eye conditions involving neovascularisation.[10] For example, in the neovascular ('wet') form of age-related macular degeneration (AMD), which accounts for 80–90 % of severe vision loss in wet AMD[11] and affects up to 4.5 million people worldwide,[12] choroidal neovascularisation (CNV) leads to leakage of fluid, protein and blood behind the retina, resulting in impaired central vision. While the aetiology of wet AMD is complex and multifactorial, CNV is a final common pathway that is directly responsible for sight loss and presents a highly relevant target for therapeutic intervention.

Approximately 8 % of all individuals with diabetes develop proliferative diabetic retinopathy (PDR).[13] As with AMD, angiogenesis-dependent PDR is a major public health problem owing to the growing incidence of diabetes and rapidly ageing populations. Thus, an effective treatment for neovascularisation will benefit thousands of PDR patients. Although rare (3.9 % of cases), secondary neovascular glaucoma (NVG) contributes disproportionately to blindness[14] and is the second most common cause for enucleations[15,16] across all eye diseases. Today's therapeutic approaches are insufficient and there is a need for better treatments.

In comparison with other retinopathies, retinopathy of prematurity (ROP) affects a relatively small number of patients.[17] However, the need for effective therapeutic strategies is critical because it affects newborns and can result in lifelong blindness. In addition, the incidence is increasing alongside the increase in the overall survival of premature babies.[18]

Finally, at the front of the eye, neovascularisation is the main risk factor for corneal graft rejection, both pre- and post-graft.[19-21] The incidence of rejection is about 15 % after the first year, increasing to around 45 % at five years.[22] Considering that more than 45,000 people in the US alone underwent a corneal transplant in 2011,[23] neovascularisation associated with corneal graft rejection (NV-CGR) can affect thousands of people worldwide. Therefore, an effective treatment to reduce neovascularisation is essential.

Anti-vascular Endothelial Growth Factors in the Treatment of Ocular Neovascularisation

Angiogenesis is a complex process controlled by numerous interacting molecules, organised as overlapping signalling pathways operating intra- and extra-cellularly. In theory, any of these interacting molecules may represent a pharmacologic target for anti- or pro-angiogenic drugs, while inhibition of one or more of the signalling pathways may help to prevent angiogenic-mediated disease. The current reference standards in anti-angiogenic therapy are drugs that directly target the vascular endothelial cell growth factor (VEGF) pathway. When VEGF, a signal protein that has a major role in stimulating vasculogenesis and angiogenesis is overimpressed, it can contribute towards disease. For example, solid cancers cannot grow without an adequate blood supply; cancer cells that can express VEGF are able to grow and metastasise. It is in the context of life-threatening oncological conditions that anti-VEGF drugs were first developed.

Anti-VEGF therapy (e.g. the monoclonal antibody ranibizumab) can be effective in ocular conditions in which neovascularisation and vascular hyperpermeability leads to blindness, especially wet AMD,[24] macular oedema, PDR and NVG.[25] Despite the success of ranibizumab in treating retinal diseases, it has the drawback that repeated intravitreal injections are typically required every 1–3 months to maintain efficacy, with a cumulative risk of adverse events. In addition, despite treatment with potent VEGF inhibitors, only 30–40 % of AMD patients gain three lines in visual acuity and roughly every sixth patient continues losing visual acuity and progresses to legal blindness.[26] Furthermore, because VEGF is involved in a wide variety of physiological processes, the long-term ocular and systemic safety of anti-VEGF agents may be of some concern;[27] angiogenesis is essential for life and long-term inhibition of neovascularisation may also interfere with healthy angiogenesis.[28] Consequently, new therapies are needed that either improve upon the therapeutic benefits of ranibizumab and/or eliminate the need for repeated intravitreal administration.

With this in mind, the development of a therapeutic strategy that clearly distinguishes between healthy and pathological angiogenesis may require other targets other than the VEGF pathway, and a different type of inhibition than direct interference with protein–protein interactions. One such target is insulin receptor substrate (IRS)-1, a molecule that is overexpressed in neovascularisation but that

does not appear to be necessary for healthy angiogenesis.[29] When the IRS-1 messenger RNA (a non-protein target) is deactivated, neovascularisation is inhibited, but physiological angiogenesis is unaffected.[30] Deactivation of the IRS-1 mRNA, and thus interference with the IRS-1 angiogenic pathway, can be achieved using an antisense oligonucleotide.

Potential of Antisense Oligonucleotides

Antisense oligonucleotides are short, single-stranded DNA polymers that can base-pair with a specific single-stranded mRNA sequence to create a DNA/RNA hybrid that is not amenable to translation, thus inhibiting gene expression. They do not interfere directly with proteins (i.e. biologically-active molecules such as growth factors, hormones, etc.) but with mRNA thus the making of proteins *per se*. Antisense oligonucleotides may thus interfere more selectively with *de novo* gene expression induced in some pathogenic processes, rather than with existing signalling pathways that are part of normal angiogenesis or other housekeeping activities in healthy cells.

To date, only one antisense oligonucleotide has been approved by the European Medicines Agency (EMA) and US Food and Drug Administration (FDA). Fomivirsen, which targets a viral mRNA and therefore interferes with viral metabolism in the narrow context of a specific viral infection, is used to treat cytomegalovirus retinitis in AIDS patients. Antisense oligonucleotides that target human (versus viral) mRNA, such as that involved with the IRS-1 angiogenic pathway may have a broader therapeutic potential. The interest in antisense oligonucleotides in disease management stems from their specific characteristics that may confer several advantages over traditional biological therapeutics. Thus, antisense oligonucleotides can be designed to be readily transported across cell membranes, allowing simplified modes of administration (including topical or pulmonary) and access to intracellular targets that protein-based biologicals cannot reach. Moreover, antisense oligonucleotides demonstrate minimal immunogenicity and so may be less likely to elicit immune responses that can occur with monoclonal antibodies, for example.[31] Finally, antisense oligonucleotide design and manufacturing processes are much simpler than those of protein-based biologicals.

Aganirsen – One Way Forward

Aganirsen (GS-101), developed by Gene Signal, is an antisense oligonucleotide that targets the IRS-1 mRNA. The drug may offer a valuable alternative and/or adjunct to anti-VEGF agents as it can be applied topically, a much simpler mode of administration rather than intravitreal injection. Furthermore, development experience to date suggests that it may have a positive long-term safety profile, as aganirsen inhibits neovascularisation without appearing to interfere with existing signalling pathways associated with healthy angiogenesis.[30] The drug has important therapeutic potential, as the number of patients that might benefit is significant, based both on the number and the nature of pathologies where aganirsen may be applicable.

Trials using aganirsen have so far focused mainly on ocular disorders because of the ability to visually assess its effect on neovascularisation, but also because of the unmet need for effective therapies in these disorders. Data from preclinical and animal models have demonstrated that aganirsen inhibits the expression of IRS-1[29,30] and significantly inhibits corneal neovascularisation[32] and lymphangiogenesis.[33] In non-human primate eyes, topical administration of aganirsen

Figure 2: Aganirsen Eye Drops Result in Significant and Near Complete Inhibition of Grade IV Experimental Choroidal Neovascularisation Development

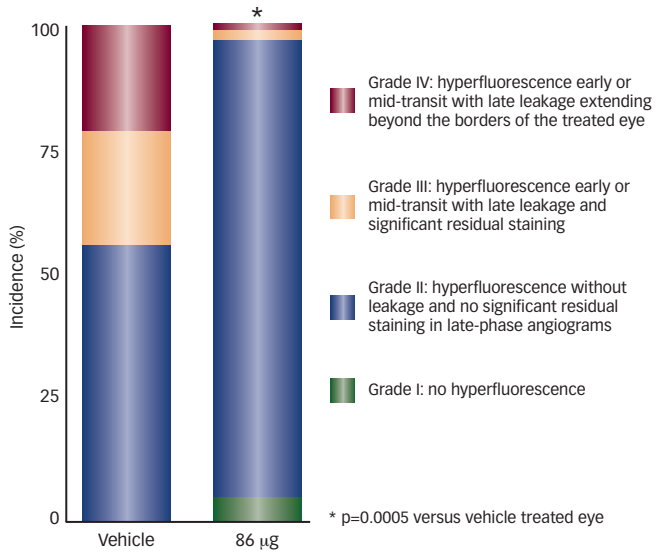
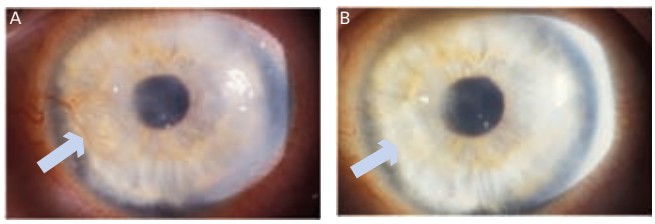


Figure 3: Aganirsen Eye Drops Inhibit Corneal Neovascularisation



A: Baseline picture before aganirsen therapy; B: Slit-lamp findings after three months of treatment – arrows indicate vascularised area.
Source: Cursiefen, et al., 2009.[34]

results in significant and near complete inhibition of laser-induced CNV lesions with the most pronounced neovascularisation compared with vehicle-treated eyes (see *Figures 1* and *2*).[30]

The most advanced clinical trials of aganirsen have been conducted in neovascularisaiton associated corenal graft rejection (NV-CGR). The phase II trial was the first randomised controlled trial (RCT) on topical inhibition of angiogenesis in the cornea.[34] When applied twice daily, aganirsen eye drops significantly inhibited progressive corneal neovascularisation (see *Figure 3*) and were well tolerated. The phase III trial is currently closing.

Phase II trials are also planned for wet AMD, NVG and PDR. Hence, treatment with aganirsen could potentially benefit thousands of patients with conditions that are dependent on neovascularisation.

Conclusions

Ocular neovascular disorders are a major cause of sight impairment worldwide. Aganirsen is a topical antisense oligonucleotide that inhibits IRS-1 and thus neovascularisation, without interfering with normal angiogenesis. Topical aganirsen significantly inhibits progressive corneal vascularisation and appears well tolerated. The results of ongoing trials for other indications will help determine its potential as a topical alternative or adjunct to conventional anti-VEGF therapy. ■

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