Delivery of anti-angiogenic molecular therapies for retinal disease

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Angiogenic diseases of the retina are the leading cause of blindness in the developed world. The development of anti-angiogenic molecular therapies has transformed the prognosis of these conditions, especially age-related macular degeneration. With these new treatments comes the new challenge of delivering an effective dosage to the retina, over a prolonged period of time and in a safe and cost-effective manner. A range of new anti-angiogenics are on the horizon, offering new and varied modes of drug delivery. In addition, a range of new sustained-release drug delivery technologies are being developed.

Retinopathy of prematurity (ROP), diabetic retinopathy (DR) and age-related macular degeneration (AMD) are three different conditions that broadly affect three different age groups. In the developed world, ROP, DR and AMD are the largest causes of blindness in infants, adults of working age and the elderly, respectively [1–3]. One feature they all have in common is the pathological proliferation of new blood vessels (neovascularization). In DR and ROP, these blood vessels originate from the retina, bleed into the vitreous and, subsequently, cause fibrosis, tractional retinal detachment and visual loss. In AMD, these blood vessels normally originate from the choroid and invade the overlying retina. Subsequent bleeding and exudation can lead to scarring and permanent loss of central vision. The neovascularization in all three conditions is driven by an angiogenic cascade, the trigger of which is believed to be relative hypoxia and oxidative stress. Vascular endothelial growth factor (VEGF-A) is a key component of this cascade but is by no means the only mediator of angiogenesis. In addition to promoting neovascularization, angiogenic factors also promote increased vascular permeability. This can lead to sight-threatening oedema in the most sensitive part of the retina, the macula. This is a complication seen in both DR and AMD, as well as in retinal vein occlusions (RVOs). Molecular treatments aimed at halting or reversing angiogenesis (anti-angiogenics) can be used to treat both neovascularization and macular oedema.

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Research into the field of ocular angiogenesis has increased rapidly, with a variety of treatments coming to clinical trial. From 2001 to 2004, 25 clinical trials involving retinal anti-angiogenic molecular therapies were registered on the ClinicalTrials.gov registry (http://clinicaltrials.gov). During the following four-year period (2005–2008), 273 clinical trials were registered, representing a more than tenfold increase (Figure 1).

The resultant new therapies targeting the VEGF-A molecule have produced a paradigm shift in the management of neovascular AMD. They have not only improved the prognosis dramatically, to a degree not seen before, but also altered patient expectations, clinical workload and the clinical costing of disease management. In addition to patient benefit, the success of the first back-of-the-eye pharmacotherapies has also triggered a massive increase in capital investment and interest from larger pharmaceutical companies. Whereas the turn of the millennium saw only a handful of biotech start-ups – such as the developer of the first anti-VEGF for ocular use, Eyetech – today there at least 30 or 40 small biotechs fuelling drug development for blinding retinal disease.

With the design of the new battery of drugs comes the question of how to deliver them to the target tissue. Delivering drugs to the retina is problematic, often resorting to invasive means such as repeated intraocular injections [4]. Newer and potentially safer methods are needed. This need has never been greater, owing to the rapid rise in new molecular entities becoming available for retinal disease.

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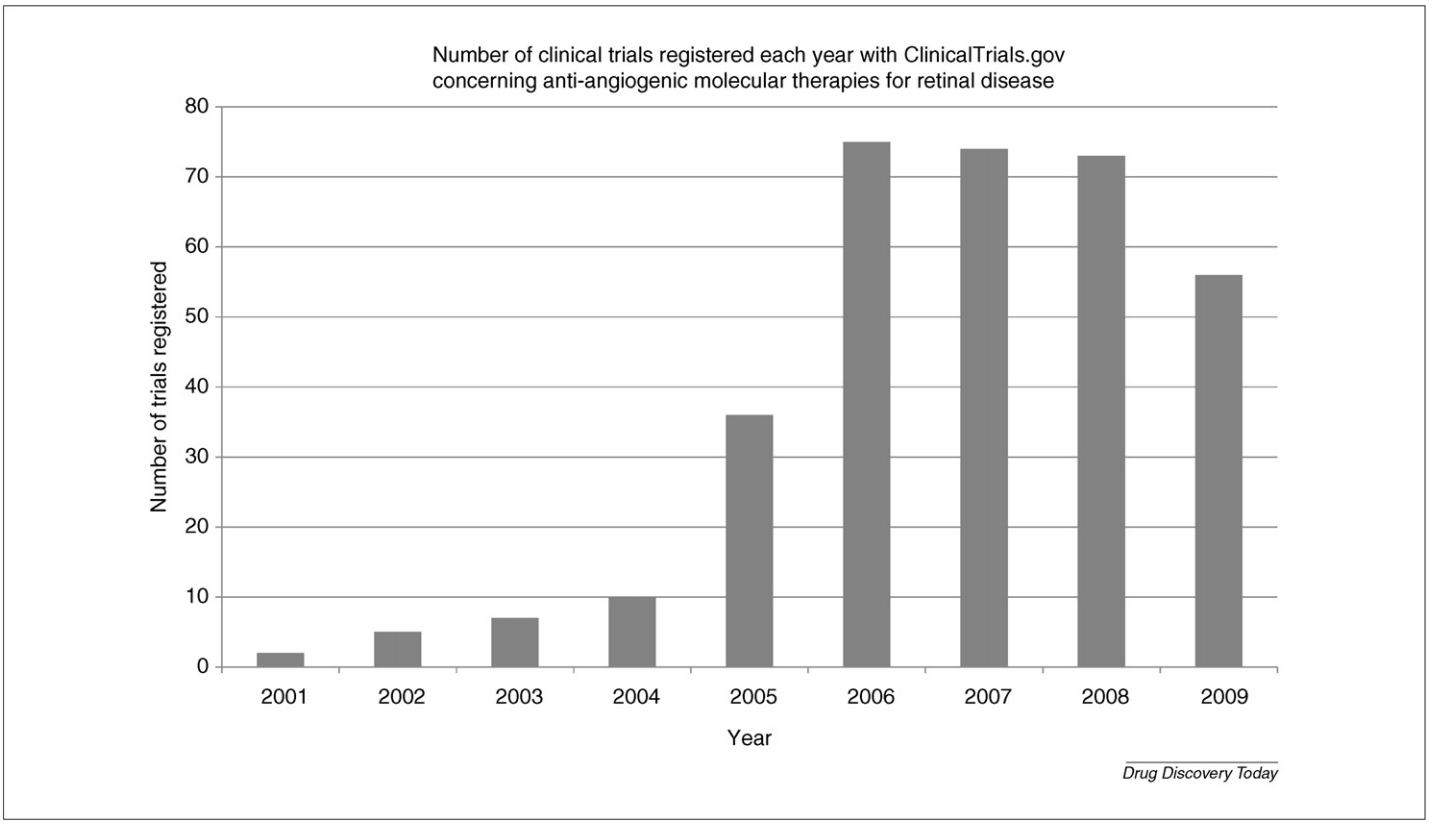

FIGURE 1

Graph displaying the number of clinical trials registered with the ClinicalTrials.gov registry (http://clinicaltrials.gov) each year between 2001 and 2009. Each entry concerns the application of an anti-angiogenic molecular therapy in the treatment of either neovascular AMD, DR, RVO or ROP.

Scope

This paper is divided into two parts. The first part will briefly highlight current and potential molecular therapies for the treatment of conditions such as AMD, DR, RVO and ROP. Only therapies currently undergoing development in phase I–IV human clinical trials will be covered. A detailed discussion of individual treatments is beyond the scope of the paper. The second part will be the main focus of the review. Molecular therapies are only useful if they can reach the target tissue; therefore, we will discuss current and potential methods for the delivery of anti-angiogenic molecular therapies in the treatment of retinal disease.

Current and potential anti-angiogenic molecular therapies

So far, only two anti-angiogenic drugs have received Food and Drug Administration (FDA) and European Medicines Agency approval for the treatment of neovascular AMD. To date, no molecular therapies have received FDA approval for the treatment of diabetic macular oedema (DMO), proliferative diabetic retinopathy (PDR) or ROP. Ozurdex (dexamethasone) has received FDA approval for the treatment of RVO-associated macular oedema. Although it is not formally considered to be an anti-angiogenic agent, it does have some intrinsic anti-angiogenic activity [5]. The main reason for including it, however, is that it is the first FDA-approved biodegradable sustained-release device for the treatment of angiogenic retinal disease. Numerous compounds are undergoing phase I–III clinical trials (Table 1), and in the meantime, many compounds are often used off-label. Here, we briefly discuss compounds that have received FDA approval and highlight certain compounds in common off-label use or in the very latest stages of development.

FDA-approved therapies

Pegaptanib (Macugen; Eyetech/Pfizer, Inc.) was the first FDA-approved anti-angiogenic treatment for neovascular AMD [6]. It is a 28-base PEGylated aptamer, which when folded correctly has a three-dimensional conformational shape that potently (dissociation constant ~50 pM) and specifically binds to the major heparin-binding isoforms of VEGF-A, blocking their action [7]. The aptamer's nucleotides have been modified to make it more resistant to degradation by endogenous endonucleases and exonucleases. The addition of polyethylene glycol (PEG) moieties, or PEGylation, increases the molecular weight and increases the half-life in the vitreous (Macugen Information Sheet, http://www.accessdata.fda.gov/drugsatfda_docs/label/2006/021756s006,s007lbl.pdf). Both of these modifications increase the biological half-life of the drug [8].

Pegaptanib was designed to be delivered by intravitreal injection every six weeks for the treatment of neovascular AMD, although its use for this condition has been largely superseded by ranibizumab [6]. Pegaptanib is perceived to have a more robust effect in DMO, however, and is in phase III clinical testing in Europe.

Ranibizumab (Lucentis; Genentech/Novartis/Roche, Inc.) was the second FDA-approved anti-angiogenic treatment for neovascular AMD. Unlike pegaptanib (which is RNA based), ranibizumab is a humanized Fab fragment of a mouse monoclonal antibody with high affinity for all isoforms of VEGF-A (unlike pegaptanib, which only binds VEGF165 and VEGF189).

TABLE 1

Anti-angiogenic molecular therapies that are currently under clinical development[a].

Disease	*Phase*	Mode of action	Name
nAMD			
	FDA√	VEGF inhibitor	Ranibizumab, pegaptanib
	P III	VEGF inhibitor	Bevacizumab, VEGF trap
	P II	Tyrosine kinase inhibitor	AL-39324, pazopanib, TG100801, vatalanib
		mTOR inhibitor	Everolimus, sirolimus
		nAChR inhibitor	Mecamylamine
		RTP801 inhibitor	PF-4523655
		Corticosteroid	Fluocinolone, triamcinolone
		NSAID	Brofenac
	P I	VEGF inhibitor (viral delivery)	AAV2-sFLT01
		PEDF inhibitor (viral delivery)	AdGVPEDF.11D
		PDGF inhibitor	E10030
		α5β1 integrin receptor inhibitor	JSM6427, volociximab
		Complement inhibitor	ARC1905, POT-4
		C-raf kinase inhibitor	iCo-007
		S1P inhibitor	iSONEP
		TORC1/TORC2 inhibitor	Palomid 529
		TNFα inhibitor	Adalimumab, infliximab
		Anti VEGF receptor vaccine	VEGF R1 & R2
PDR			
	P III	VEGF inhibitor	Bevacizumab, ranibizumab
		PKCβ inhibitor	Ruboxistaurin
		Somatostatin analogue	Octreotide
	P II	Corticosteroid	Triamcinolone
		MMP inhibitor	Doxycycline
DMO			
	P III	VEGF inhibitor	Ranibizumab, pegaptanib, bevacizumab
		Corticosteroid	Fluocinolone, triamcinolone, dexamethasone
		PKCβ inhibitor	Ruboxistaurin
		Somatostatin analogue	Octreotide
	P II	VEGF inhibitor	VEGF trap
		mTOR inhibitor	Sirolimus
		nAChR inhibitor	Mecamylamine
		RTP801 inhibitor	PF-4523655
		TNFα inhibitor	Infliximab
		NSAID	Nepafenac
	P I	TNFα inhibitor	Adalimumab
		NSAID	Brofenac
		VEGF inhibitor	MP0112
RVO			
	FDA√	Corticosteroid	Dexamethasone
	P III	VEGF inhibitor	Bevacizumab, ranibizumab, VEGF Trap
		Corticosteroid	Triamcinolone
	P II	VEGF inhibitor	Pegaptanib
		Corticosteroid	Fluocinolone
	P I	Plasma kallikrein inhibitor	Ecallantide
ROP			
	P II	VEGF inhibitor	Bevacizumab

[a] Therapies are grouped according to the latest phase of clinical development (FDA approval, phase I–III clinical trial) and their mode of action. Information is courtesy of the ClinicalTrials.gov registry (http://clinicaltrials.gov) and was updated on 1 February 2010. To the best of the authors' knowledge, all the therapies described are still under development; however, development of certain drugs might have been cancelled without public knowledge.

Abbreviations: DMO, diabetic macular oedema; FDA√, Food and Drug Administration approved; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; nAChR, nicotinic acetylcholine receptor; nAMD, neovascular age-related macular degeneration; NSAID, non-steroidal anti-inflammatory drug; PDGF, platelet-derived growth factor; PDR, proliferative diabetic retinopathy; PEDF, pigment epithelium-derived factor; PKCβ, protein kinase C beta; P III, phase III; ROP, retinopathy of prematurity; RVO, retinal vein occlusion; S1P, sphingosine-1-phosphate; TNFα, tumour necrosis factor alpha; VEGF, vascular endothelial growth factor.

Ranibizumab was designed to be delivered by intravitreal injection every four weeks [9,10], although current practice is to administer three doses at four-week intervals, then to administer according to clinical need [11]. Importantly, it was the first treatment for neovascular AMD that resulted in a statistically significant improvement in visual acuity in all lesion subtypes [9,10]. Although no head-to-head trial was performed against pegaptanib, better perceived clinical outcomes with ranibizumab make blockage of all VEGF-A isoforms the current strategy of choice for AMD. Ranibizumab is also in clinical testing for PDR, DMO and RVOs. Results seem promising in the short term; however, in the long term, it needs to show benefit over and above that of laser therapy. Although laser therapy is potentially destructive, side-effects of long-term

VEGF blockage, particularly in ischaemic diabetic retina, are not fully understood.

Off-label therapies

Bevacizumab (Avastin; Genentech/Roche, Inc.) is the monoclonal antibody from which ranibizumab was derived. Like ranibizumab, bevacizumab binds all isoforms of VEGF-A. It was first licensed by the FDA for use in metastatic colon cancer.

The vitreous half-life of bevacizumab is longer than that of ranibizumab [12]. Bevacizumab, once alliquoted into unit doses, is considerably cheaper than ranibizumab per unit dose. As a result, it has been extensively used, off label, for the treatment of neovascular AMD. A head-to-head trial between bevacizumab and ranibizumab is currently underway both in the USA (CATT; http://clinicaltrials.gov/ct2/show/NCT00593450) and in the UK (IVAN; http://www.ivan-trial.co.uk/Default.aspx). These trials will provide high-quality efficacy data on bevacizumab.

Bevacizumab is currently the only anti-angiogenic molecular therapy under clinical trial for the treatment of ROP. VEGF is important in developmental angiogenesis. Ongoing clinical trials will need to assess whether temporary VEGF blockade in premature babies has any untoward effects on developmental angiogenesis.

Promising treatments in end stage of development

VEGF Trap is a recombinant protein made up of the domains of VEGF receptors 1 and 2 and the Fc portion of human IgG [13]. The Fc portion increases the intravenous circulating half-life. VEGF Trap has a very high affinity for all isoforms of VEGF-A, as well as other VEGF family members [14]. This broader range of binding is a key difference between it and ranibizumab. The administration of VEGF Trap for the treatment of neovascular AMD initially concentrated primarily on intravitreous delivery. Dose-limiting toxicity was seen during a phase I trial of intravenous administration for the same condition [15]. The phase II study into intravitreal use of VEGF Trap for the treatment of neovascular AMD (Clinical Evaluation of Anti-angiogenesis in the Retina Study, or CLEAR-IT-2) has recently reported final results showing a statistically significant reduction in retinal thickness, a statistically significant improvement in visual acuity and an acceptable safety profile following a 2.0 mg dosing regime (http://www.viva.vita.bayerhealthcare.com/index.php?id=36&tx_ttnews[tt_news]=12724&cHash=81f109c102).

Two phase III clinical trials are underway (VIEW-1 in the USA and Canada and VIEW-2 in Europe, Asia-Pacific, Japan and Latin America). These non-inferiority studies aim to compare efficacy of VEGF Trap against ranibizimab. Study completion is expected in 2012 and 2011, respectively (http://clinicaltrials.gov/ct2/show/NCT00509795; http://clinicaltrials.gov/ct2/show/NCT00637377). The effect of VEGF Trap on DMO is in phase II clinical testing (http://clinicaltrials.gov/ct2/show/NCT00789477). Table 1 also includes other compounds in earlier stages of clinical development. Delivering these to the retina will be the next challenge, should the trials prove to be successful.

Issues regarding delivery of anti-angiogenic molecular therapies

The eye is a structurally unique organ. As a result, drug delivery can be problematic. The retina receives a rich blood supply but is protected by a blood–retinal barrier at the endothelial cell and retinal pigment epithelial (RPE) interfaces. The retina lies some distance from the ocular surface, where topical drugs are administered (Figure 2). Penetration of drugs into the eye is limited by structural and functional barriers. Structural barriers, such as corneal, conjunctival and scleral tissue, limit drug diffusion. Functional barriers, such as rapid drug clearance by conjunctival vessels, remove drugs before they can reach the target tissue.

To design an effective treatment, one needs to consider not only pharmacodynamics (what the drug does to the body) but also pharmacokinetics (what the body does to the drug). The chemical structure of a drug affects not only its pharmacodynamic properties but also its pharmacokinetic properties. Two important characteristics of the chemical structure are molecular weight (MW) and lipophilicity [16,17]. In general, ocular penetration is inversely proportional to increasing MW and proportional to increasing lipophilicity. Therefore, inherently small lipophilic compounds penetrate the eye more easily. Examples of this include the aromatic compound pazopanib (GlaxoSmithKline, Inc.), which is delivered topically in a current trial for the treatment of neovascular AMD (http://www.clinicaltrials.gov/ct2/show/NCT00612456). Table 2 displays the mode of delivery in humans for a range of compounds (under clinical trial) alongside their MW. Small molecules tend to be delivered topically or transsclerally because they can penetrate the eye more easily. Larger hydrophilic molecules, such as the currently licensed VEGF-A inhibitor ranibizumab, tend to be delivered into the eye via an invasive intravitreal injection because of poor ocular penetration. Because the majority of ocular angiogenic diseases are chronic in nature, this invasive mode of drug delivery can be repeated many times. Repeated treatment incurs financial and manpower costs, and each injection presents a small risk of a blinding complication (intraocular infection or retinal detachment). In the VISION study assessing pegaptanib, the rates per injection of intraocular infection and retinal detachment were 0.16% and 0.08%, respectively [6]. In the MARINA study assessing ranibizumab, the rate of presumed intraocular infection per injection was 0.05%. With each injection, the cumulative risk increases. Over the two years of the MARINA study, the cumulative rate increased to 1% [9]. Therefore, issues arise concerning not only how to deliver the chosen drug to the target area but also how to do it safely and cost-effectively over a prolonged period of time.

Intravitreal delivery

Intravitreal delivery has proved to be the delivery method of choice for the current approved therapies for AMD. This bypasses both the blood–retinal barriers and structural/functional barriers. In sight-threatening disorders, physicians can also be sure that compliance is not an issue because the drug is delivered by the physician. There are many techniques under investigation that aim to enhance the duration of action following intravitreal drug delivery, leading to a reduced frequency of intervention.

Intravitreal injection

MW and lipophilicity influence the penetration of drugs into the eye. These characteristics of a drug also influence its half-life in the vitreous [18,19]. Small drugs escape from the vitreous more easily than large ones. This has led to small molecules, such as the

TABLE 2

Mode of delivery of anti-angiogenic molecular therapies in human clinical trials[a].

Structure	Name	Mode of action	MW (kDa)	Top	TS	ITV Inj	ITV Imp	Sys
Amino acid based								
Adenoviral vector	AdGVPEDF.11D	PEDF inhibitor	NA			●		
Adeno-associated viral vector	AAV2-sFLT01	VEGF inhibitor	NA			●		
Cyclic peptide	POT-4	Complement inhibitor	1.74			●		
DARPin protein	MP0112	VEGF inhibitor	NA			●		
Fab fragment	Ranibizumab	VEGF inhibitor	48			●		
Monoclonal antibody	Adalimumab	TNFα inhibitor	144		●	●		●
	Bevacizumab	VEGF inhibitor	149			●		
	Infliximab	TNFα inhibitor	144			●		
	iSONEP	S1P inhibitor	NA			●		
	Volociximab	α5β1 integrin receptor inhibitor	146			●		
Peptide	VEGFR1 and R2	Anti VEGFR vaccine	NA			●		●
	Ecallantide	Plasma kallikrein inhibitor	7.05					
Octapeptide	Octreotide	Somatostatin analogue	1.02					●
Recombinant protein	VEGF Trap	VEGF inhibitor	115			●		●
Nucleic acid based								
PEGylated aptamer	ARC1905	Complement inhibitor	NA			●		
	E10030	PDGF inhibitor	NA			●		
	Pegaptanib	VEGF inhibitor	~50			●		
Oligonucleotide	PF-4523655	RTP801 inhibitor	~13.3			●		
	iCo-007	C-raf kinase inhibitor	NA			●		
Cortisol based								
Corticosteroid (synthetic)	Dexamethasone	Corticosteroid	0.39		●	●	●	
	Fluocinolone	Corticosteroid	0.45				●	
	Triamcinolone	Corticosteroid	0.43				●	
Other synthetic organic compounds								
Amidated bicyclo hydrocarbon	Mecamylamine	nAChR inhibitor	0.17	●				
Aromatic compound	AL-39324	AL-39324	0.38	●	●	●		●
	Bromfenac	NSAID	0.33	●		●		●
	Doxycycline	MMP inhibitor	0.44	●				
	Nepafenac	NSAID	0.25	●				
	Palomid 529	TORC1/TORC2 Inhibitor	0.41					
	Pazopanib	Tyrosine kinase inhibitor	0.47					
	TG100801	Tyrosine kinase inhibitor	~0.57					
	Vatalanib	Tyrosine kinase inhibitor	0.35					
Macrolide	Everolimus	mTOR inhibitor	0.96		●	●		●
	Sirolimus	mTOR inhibitor	0.91					●
Macrocyclic bisindolylmaleimide	Ruboxistaurin	PKCβ inhibitor	0.47					●
Pyrrolidine derivative	JSM6427	α5β1 integrin receptor inhibitor	NA			●		

[a] Therapies are grouped according to their chemical structure because this is the feature most likely to influence their mode of delivery into the eye. A brief description of their mode of action is given. Molecular weight is presented alongside mode of drug delivery. Information is courtesy of the ClinicalTrials.gov registry (http://clinicaltrials.gov) and was updated on the 1 February 2010. To the best of the authors' knowledge, all the therapies described are still under development; however, development of certain drugs may have been cancelled, without public knowledge.

Abbreviations: ITV Inj, intravitreal injection; ITV Imp, intravitreal implant; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; MW, molecular weight; nAChR, nicotinic acetylcholine receptor; NSAID, non steroidal anti-inflammatory drug; PDGF, platelet derived growth factor; PEDF, pigment epithelium-derived factor; PKCβ, protein kinase C beta; S1P, sphingosine-1-phosphate, Sys, systemic; Top, topical; TS, transscleral; TNFα, tumour necrosis factor alpha; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

aptamer pegaptanib, being PEGylated, to increase their MW. Other VEGF-A inhibitors have the intrinsic advantage of having a chemical structure with a high MW (bevacizumab, ranibizumab and VEGF Trap). VEGF Trap has an intravitreal half-life in the rabbit eye of 4.79 days, compared with 2.88 days for ranibizumab and 4.32 days for bevacizumab (Regeneron Pharmaceuticals, Tarrytown, New York, USA, unpublished) [12]. This is longer than expected considering its MW lies between that of ranibizumab and bevacizumab (Table 2). There is another characteristic of VEGF Trap that might influence its clinical ability to maintain VEGF-A blockade after intravitreal injection: the dissociation constant (Kd). VEGF trap has a dissociation constant for all VEGF-A isoforms of <1 pM (compared with 0.15 nM and 1.1 nM for ranibizumab and bevacizumab, respectively) [15,20,21]. Therefore, it shows an approximately 150-fold and 1100-fold higher affinity for VEGF-A than ranibizumab and bevacizumab, respectively. Mathematical modeling, taking into account the dissociation constants of VEGF Trap and ranibizumab, shows that 79 days after VEGF Trap injection, the intravitreal VEGF-binding activity should be comparable to ranibizumab at 30 days [22]. Theoretically, VEGF Trap might be given less frequently while maintaining efficacy. Whether this theory equates into practice is dependent on the outcome of clinical trials.

Despite modifications and variations in the chemical structure promoting an increased intravitreal half-life, it will still be short when compared with the duration of disease. Therefore, other intravitreal delivery techniques, which deliver the chosen drug for a longer period of time, have been developed.

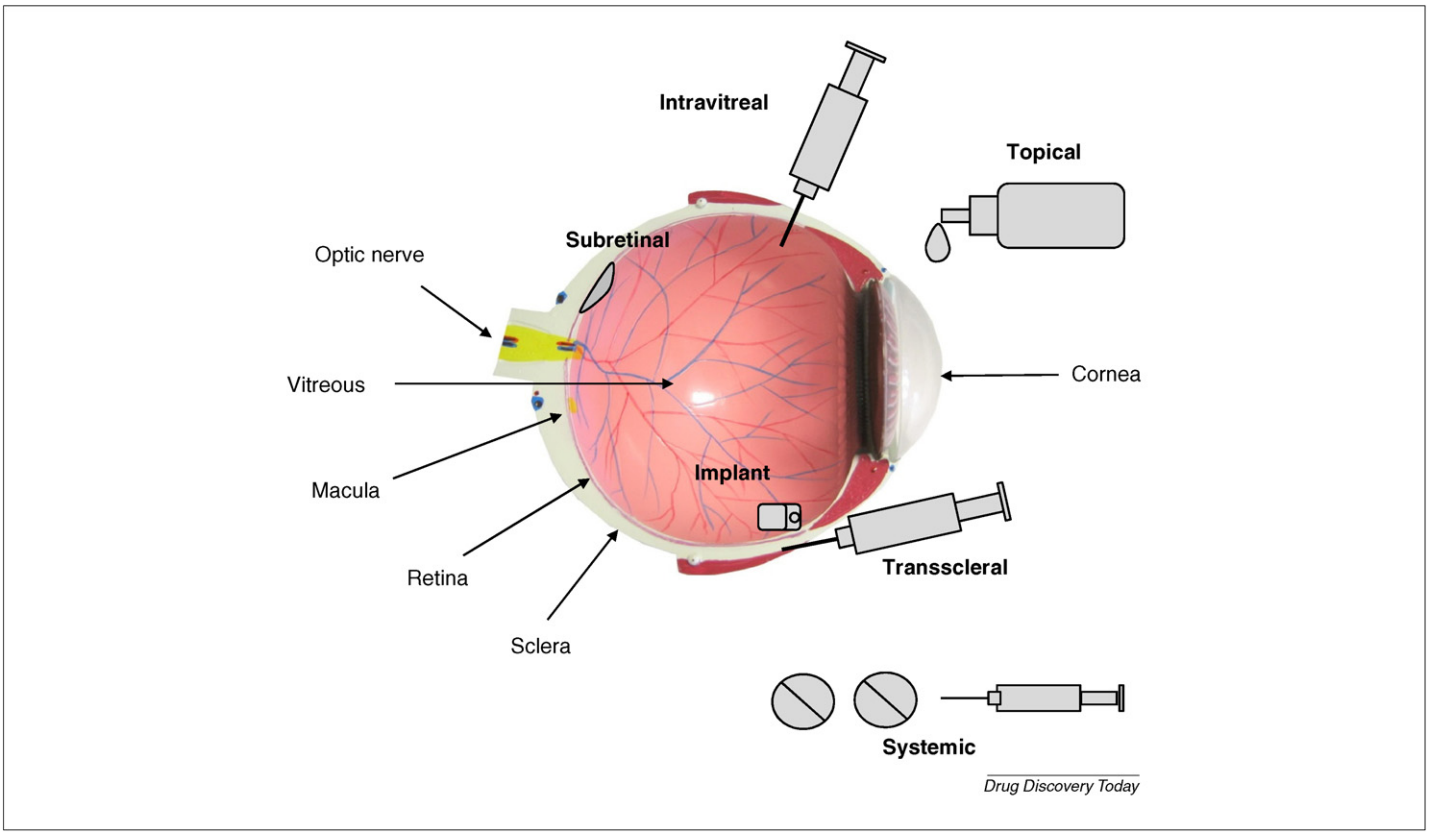

FIGURE 2

Diagram showing the structure of the eye, alongside a variety of different methods of delivering anti-angiogenic molecular therapies to the retina.

Non-biodegradable implants

Reservoir non-biodegradable implants contain a drug core surrounded by a permeable membrane (e.g. polyvinyl alcohol). This is then surrounded by a semipermeable membrane (e.g. ethylene vinyl acetate polymer or silicone) containing an aperture allowing release of the drug. They have the ability to release a steady amount of drug (zero-order kinetics) for at least one year [23]. The first implant of this type to be approved was Vitrasert™ (Bausch & Lomb, Inc.) [24]. This ganciclovir-releasing implant was extensively used in the treatment of cytomegalovirus retinitis. The design of Retisert™ (Bausch & Lomb, Inc.) is very much along the lines of the Vitrasert™ implant. It releases fluocinolone acetonide and is licensed for the treatment of chronic non-infectious uveitis affecting the posterior segment of the eye. It is also under trial for the treatment of DMO [25] and macular oedema caused by RVO (http://clinicaltrials.gov/ct2/show/NCT00168324).

Surgical delivery of such an implant involves a pars plana incision and anchoring to the sclera via a suture. Compared with a single intravitreal injection, this procedure is more prone to complications and more difficult to perform [26,27]. Issues with regards to implantation can be reduced by the use of injectable reservoir implants. Iluvien™ (formerly Medidur™; Alimera Sciences/pSivida, Inc.) also releases fluocinolone acetonide and is an example of such an injectable reservoir implant.

Reservoir-based delivery is an attractive system for the delivery of anti-angiogenic factors because of its steady rate of drug delivery over a prolonged period of time.

Newer designs of non-biodegradable implants are coming through development. An example is the Ivation™ implant (SurModics, Inc.). This is a non-ferrous metallic implant shaped like a corkscrew. The corkscrew region is coated with a durable polymer matrix especially suited to the delivery of hydrophobic molecules. The implant traverses the sclera, with the corkscrew region lying in the vitreous cavity. This novel design makes it relatively easy to insert and remove. Phase II trials assessing a triamcinolone-loaded Ivation™ implant are underway for the treatment of DMO (http://clinicaltrials.gov/ct2/show/NCT00692614).

A key requirement when choosing a drug to use with a non-biodegradable implant is the long-term stability of the drug. Synthetic organic compounds such as dexamethasone, fluocinolone and sirolimus have proved to be sufficiently stable for long-term release; however, anti-angiogenic agents made up of amino or nucleic acids can become unstable while in the implant. They are particularly vulnerable to attack by endogenous nucleases and proteases.

Biodegradable implants

Another strategy for prolonging the release of anti-angiogenic agents would be to incorporate them into biodegradable implants. Unlike non-biodegradable implants, surgical removal or permanent ocular residence of the spent implant is not an issue. Implants can be manufactured from a variety of compounds. Poly-lactic acid (PLA) and copolymers with glycolic acid, making poly(lactic-co-glycolic acid) (PLGA), have been widely investigated for this task. They break down into lactic and glycolic acids, both of which are metabolized via the Krebs cycle to carbon dioxide and water [28]. The drug of choice is dispersed within this matrix and is released via diffusion and after degradation of the matrix. As a result, drug release does not

follow zero-order kinetics, although better control of release can be achieved through blending PLAs of different MWs [29].

These implants tend to be solid in nature, although more viscous materials such as poly(ortho esters) (POE) also exist. POE offers the attractive ability to inject the matrix, with the formation of a ball-like structure in the vitreous. The release of drug from POE only occurs after biodegradation at the polymer surface. This is in contrast to PLA and PLGA matrices, in which biodegradation occurs throughout the matrix. As a result, zero-order kinetics is achieved with the POE implant [24].

Biodegradable implants can be fashioned into a variety of shapes and sizes. Single pellet implants can be injected into the eye. Ozurdex™ (formerly Posurdex™; Allergan, Inc.) is an example of one such implant, being made of PLGA. It releases dexamethasone over a prolonged period of time and has been FDA approved for the treatment of RVO. Another option is to formulate microspheres (1–1000 μm diameter) or nanospheres (1–1000 nm diameter). These also have the advantage of being injectable, although they might cloud the vision temporarily [30]. Clouding tends to be less of a problem with microspheres because their larger size allows them to sink to the bottom of the vitreous over time. PLA microspheres have been shown to be retained in the vitreous for as long as 1.5 months in a rabbit model [31], enabling sustained drug delivery over a prolonged period [30,32]. Triamcinolone-loaded PLGA microspheres (RETAAC) have already reached phase I/II trials for the treatment of refractory diffuse DMO [33].

There has also been some success in using this system to release both amino-acid and nucleic-acid-based angiogenic molecules. Short (40 amino acid) therapeutically active fragments of the potent endogenous anti-angiogenic protein pigment epithelium-derived factor (PEDF) have been loaded successfully into nanospheres [34]. The oligonucleotide pegaptanib sodium has been loaded successfully in a PLGA microsphere matrix. Release was shown to occur over 4–6 months after intravitreal injection [35]. This data, using both PEDF and pegaptanib, supports the principle that both amino-acid and nucleic-acid-based compounds can be stably incorporated into biodegradable implants. This not only prolongs drug delivery but also protects embedded amino or nucleic acid polymers from degradation by endogenous enzymes. This further prolongs the drugs' stability and duration of action [36]. Building on this basic research, Genentech/Roche, Inc. have recently signed an agreement with SurModics, Inc. to use their proprietary biodegradable microparticle drug delivery system to develop and commercialize a sustained drug delivery formulation of ranibizumab.

Although biodegradable delivery methods seem plausible, problems might arise during preparation. Many of the modern anti-angiogenics are amino-acid or nucleic-acid-based molecules with specific and potentially labile conformational structures. The challenge is incorporating such delicate molecules in the carrier matrix while maintaining their functional activity. Performing this on a large scale, while maintaining appropriate quality control, is the challenge in bringing suitable biodegradable delivery systems to market.

Topical and transscleral delivery

The delivery of anti-angiogenic compounds to the choroid and retina without disrupting the integrity of the globe is highly desirable. The complication profile of topical or transscleral delivery, compared with intravitreal drug delivery, is less severe. We have grouped topical and transscleral delivery methods together because, in general, topically delivered anti-angiogenics penetrate the eye to reach the retina via the transscleral route [37].

In the past, the delivery of topical anti-angiogenic medication for the treatment of retinal disease seemed impossible. Patient compliance issues aside, eye drops often remain on the surface of the eye for no more than five minutes, and less than 5% of the instilled drug is absorbed into the eye. The majority is absorbed into the systemic circulation, via the conjunctival and nasopharyngeal mucosa [38]. The drug concentration levels between the cornea and retina differ by several orders of magnitude. This is predominantly because of various static and dynamic barriers that prevent active drug from reaching the retina [4].

More recently, there has been hope that the treatment of diseases such as neovascular AMD might be possible with the application of eye drops. Compounds such as pazopanib, TG100801 and mecamylamine are going through phase II trials assessing topical application. TG100801 has been shown to reach the retina predominantly via the transscleral route [37]. It is not surprising when one considers that the conjunctival surface occupies a considerably greater surface area than the corneal surface. It is also more closely opposed to the retina and has an epithelial structure which is more leaky [39,40]. Pazopanib, TG100801 and mecamylamine also have inherent properties, related to their chemical structure, that make topical delivery possible. They are small, lipophilic compounds, enabling penetration across the lipophilic epithelial cell membranes (transcellular) and between epithelial cells (paracellular). This degree of absorption makes topical drug delivery a viable option.

In addition to the inherent characteristics of the drug enabling absorption after topical delivery, there are characteristics of the carrier that can promote increased absorption. Phase-changing polymers, which have a role in intravitreal and transscleral extended release, might also prove beneficial in topical delivery. Such polymers exist in a liquid form under certain conditions, while adopting a gel matrix if there is a change in temperature, pH or ion concentration [4]. The conversion of a liquid to a gel upon contact with the surface of the eye might enhance and prolong delivery. Positively charged liposomes and PEGylated nanospheres have also been shown to interact with the negatively charged mucinous corneal layer, promoting retention [36]. As with phase-changing polymers, this characteristic is only of benefit if significant amounts of the drug can leave the liposome, microsphere or gel while it is *in situ*. If the drug is retained in the carrier for longer than the carrier is retained on the globe, then drug absorption will still be poor [36,41].

Absorption of topically delivered drugs across the sclera is hindered broadly by three barriers. They have been defined as static, dynamic and metabolic [42]. Static barriers consist of the anatomical layers of the globe (conjunctiva and sclera). Fortunately, the sclera is well hydrated and is relatively permeable, even to large hydrophilic compounds. However, for large MW compounds, scleral permeability has been shown to decrease with increasing age [43]. Because angiogenic ocular disease is often age related, this decline in permeability might prove relevant when considering transscleral delivery of large anti-angiogenic compounds. Dynamic barriers include the removal of drug from

the episcleral space by conjunctival blood and lymphatic vessels [44–47]. This is the most prominent dynamic barrier to transscleral delivery. One technique to overcome this involves the design of episcleral exoplants [45,48]. These devices allow access of the drug to the sclera surface, while hindering access to the conjunctiva through the use of an impermeable membrane. As a result, more drug enters the eye transsclerally and less is absorbed systemically. Intrascleral and suprachoroidal delivery are two other methods of reducing this problem [49–52]. Metabolic barriers involve drug degradation by endogenous enzymes, before reaching the target tissue. Fortunately, the sclera is relatively metabolically inert with few proteolytic enzymes or protein-binding sites that could degrade or sequester drugs.

Topical absorption of larger charged hydrophilic molecules, such as oligonucleotides, shows some degree of absorption through the conjunctival epithelium. This absorption is not enough to substantially penetrate the globe without the assistance of iontophoresis or bypassing of the lipophilic conjunctival barrier through the subconjunctival placement of the drug [53]. Subconjunctival placement of the PEGylated aptamer pegaptanib, in PLGA microspheres, not only enabled effective transscleral diffusion but also preserved the functional activity of the drug [54].

With regards to the transscleral delivery of amino-acid-based compounds, agents as large as IgG (150 kDa) have been shown to enter the eye via this method, while retaining functional activity [16]. As the MW of the molecule in question decreases, the scleral permeability increases exponentially [16]. It would seem, therefore, that the transscleral delivery of smaller anti-angiogenic proteins such as ranibizumab might be a viable option and worth further investigation.

Rather than relying on passive diffusion of molecules across the sclera, therapeutic agents can also be actively transported. A small electrical current is capable of moving charged molecules into the eye. This technique is called iontophoresis. Nucleic-acid-based molecules, such as oligonucleotides, are inherently negatively charged, making them ideal for this application. Indeed, intraocular penetration of an oligonucleotide has been demonstrated using this technique in an animal model. The functional activity of the oligonucleotide was maintained after iontophoresis [55].

Modern techniques, using lower currents, have an improved safety profile, are non-invasive and are more convenient [56,57]. Although they are less invasive than intraocular injections, the duration of drug action is unchanged; therefore, the financial costs surrounding frequent administration will remain high. Recently, Eljarrat-Binstock *et al.* [58] published on the delivery of negatively charged nanospheres via iontophoresis. Delivery of negatively charged nanospheres, loaded with an anti-angiogenic therapeutic agent, might be a plausible method of non-invasively delivering sustained-release drugs to the posterior segment. This might reduce not only the frequency of administration but also the costs.

Gene therapy

Gene therapy involves the use of genes to treat disease. This technique can involve the replacement of a faulty gene or the insertion of a new gene. Angiogenic disease can be addressed by the insertion of genes that encode anti-angiogenic proteins. This aims to tip the balance away from angiogenesis and towards neovascular regression. The delivery of gene therapy is often invasive, involving an intravitreal or subretinal injection. However, the extended duration of gene expression means that if successful, the procedure would be performed infrequently. Therefore, gene therapy should be considered as a sustained-release form of drug delivery.

A key step to gene therapy is enabling the genetic material to gain access to the target cell. The process of gaining access can involve the use of viral-based and non-viral-based techniques. In general, non-viral-based techniques are easier to prepare, have no limit to the size of the delivered gene and are less likely to provoke an immune response [4]. However, they generally have a lower transfection rate and a shorter duration of expression than viral techniques. Examples of non-viral techniques include combining genetic material with PEG-substituted lysine peptides, polyamidoamine dendrimers (tree-like branched macromolecules), liposomal formulations and PLGA nanospheres [53,59]. After intravitreal injection, plasmid-laden PLGA nanospheres have been shown to not only reach RPE cells but also transfect them effectively [60]. Endocytic absorption of these particles enables plasmid entry into the cells. Despite a wide variety of preclinical data on non-viral-based techniques, their application in human clinical trials has been limited to the delivery of siRNA. These oligonucleotides induce gene silencing after entry into the cell. With regards to the delivery of new genetic material into a cell, clinical trials have focused exclusively on viral-based techniques. Two viruses have shown particular promise in the delivery of anti-angiogenic DNA: adeno-associated virus (AAV) and adenovirus. AAV vectors efficiently and stably transduce photoreceptors and RPE cells. They have low immunogenicity and gene transcription persists for a long period of time. AAV has been shown to be capable of suppressing CNV through the subretinal delivery of the VEGF-A inhibitor sFlt-1 (soluble VEGFR-1) [61]. More recently, AAV vectors have been shown to deliver chimeric versions of sFlt-1 (Genzyme, Inc.), which suppress angiogenesis in a model of ROP. Delivery was via intravitreal injection (as opposed to the more technically challenging subretinal injection) with gene expression (transduction) in retinal ganglion cells for at least 12 months [62]. Phase I human trials are already underway to assess intravitreal AAV delivery of sFlt-1 for the treatment of neovascular AMD (http://clinicaltrials.gov/ct2/show/NCT01024998).

In contrast to the low immunogenicity of AAV vectors, adenoviral vectors have a higher risk of immunogenicity and as a result have a shorter duration of expression [63]. However, adenoviral vectors are capable of carrying larger genes. Adenoviral vectors have been shown to be capable of carrying genetic material encoding VEGF Trap and the potent angiostatic protein PEDF [64]. During the first human trial of recombinant PEDF introduced via the adenoviral vector AdPEDF.11 (Genvec, Inc.), the intravitreal route was chosen [65]. Results suggested that intravitreal delivery is relatively well tolerated with the anti-angiogenic effect of PEDF persisting for several months [65]. Of interest is the fact that AdPEDF.11 has been shown to suppress laser-induced CNV to statistical significance, when delivered not only via subretinal and intravitreal injection but also by periocular injection in a mouse model. In the case of periocular delivery, the transduced cells were located in the periocular region and the recombinant PEDF diffused through the sclera into the eye [66–68]. This viral vector has provided valuable information with regards to

where to deliver gene therapy for the treatment of ocular angiogenesis.

One problem with periocular gene delivery is that high amounts of protein expression are needed because only a limited amount will diffuse into the eye and reach the target tissue; however, delivery of gene therapy to the subretinal space enables the transfection or transduction of cells in close proximity to the neovascular complex. As a result, lower protein expression rates are necessary to achieve the required therapeutic concentration at the target site [69]. This method of delivery has been demonstrated to have no immediate adverse effects in phase I trials of gene therapy for Leber's congenital amaurosis. Delivery of healthy RPE65 cDNA into the subretinal space via an AAV vector was shown to be a viable option [70–72]. Effective transduction in cases of inherited retinal dystrophies paves the way for the use of similar techniques for the treatment of ocular angiogenesis.

In chronic diseases such as diabetes and AMD, it might be desirable to express therapeutic proteins over a long period of time. However, the unregulated and persistent genetic expression over long periods of time raises potential safety concerns. Unlike an intravitreal implant, genetically altered cells cannot be easily removed, should the released compound display toxic side-effects. In addition, angiogenic diseases display remitting and relapsing characteristics. It would be ideal if expression occurred only if and when it was needed. With regards to non-viral transfection, the short duration of expression means that any side-effects will be short-lived. With regards to more stable expression using AAV vectors, it is possible to control gene expression via activation of upstream promoters, sensitive to tetracycline or rapamycin [73,74]. Administration of these exogenous compounds activates gene expression. Without these drugs the genes are silent. Another option would be the use of a promoter sensitive to local hypoxia. The incorporation of a hypoxia-response element into the promoter has been shown to enable gene upregulation only at hypoxic locations such as CNV [75]. This might be ideal for the treatment of angiogenesis. Hypoxia-induced release of anti-angiogenic factors, both preceding and preventing the onset of the sight-threatening neovascularization, could be a powerful way of preventing future retinal injury.

There are techniques of combining gene therapy with the placement of an intravitreal implant. Encapsulated cell technology (ECT) involves the placement of a non-biodegradable implant in the anterior vitreous. Like the non-injectable reservoir implants described above, it is implanted via a pars plana incision and attached to the sclera. However, instead of a drug core, the semipermeable membrane surrounds living cells, held in a supportive matrix. These cells are robust enough to survive long term in the implant and have been genetically engineered (through the insertion of specific genes) to continuously produce a therapeutic agent of choice. The function of the semipermeable membrane is threefold: to allow release of the therapeutic agent, to allow oxygen and nutrients to be absorbed, and to protect the cells from the host's immune system. A phase I trial has tested the safety of ECT for the treatment of photoreceptor degeneration in humans [76]. Ciliary neurotrophic factor (CNTF) was released for a period of six months. Animal studies have demonstrated the production and release of other agents, such as fibroblast growth factor-2 [77]. Both CNTF and fibroblast growth factor-2 have no anti-angiogenic role. However, they demonstrate the ability of ECT to produce amino-acid-based therapeutic agents. This shows promise for the treatment of angiogenesis because many anti-angiogenics are proteins (e.g. VEGF-A inhibitors and soluble VEGF receptors). Freshly synthesized protein factors, released at the target site, have been shown to be more effective than purified recombinant factors [78]; therefore, a lower total dose is required. The issue of controlling drug release according to clinical need might be overcome by using external agents that can upregulate or downregulate the expression of the therapeutic gene in the implanted cells [73,74].

Systemic delivery

Systemic delivery of anti-angiogenics for the treatment of ocular disease is beset with issues concerning systemic side-effects of treatment. Because of the blood–retinal barrier, considerable systemic drug loading is required to achieve therapeutic intraocular penetration. Aromatic compounds, such as tyrosine kinase inhibitors, are capable of penetrating the blood–retinal barrier because of their small lipophilic structure enabling transcellular migration. Systemic delivery of such treatments has been trialled (http://www.novartisclinicaltrials.com/webapp/clinicaltrialrepository/public/login.jsp). Owing to their broad spectrum of action, not only in the eye, systemic delivery of such drugs was not without systemic side-effects. Systemic delivery of larger compounds such as VEGF Trap was also associated with marked systemic side-effects [15]. In addition to side-effects, angiogenesis has an important role systemically, early in life during development and later during vascular remodelling. It would, therefore, seem prudent to deliver ocular anti-angiogenics via local rather than systemic means, if at all possible.

Concluding remarks

The prognosis of angiogenic retinal diseases such as neovascular AMD and DR has improved substantially in recent years. Current treatment with ranibizumab has controlled neovascular AMD to a degree never seen before, and many new molecular therapies are on the horizon. Despite these advances, the current technique of delivering drugs by frequent intravitreal injection is crude, time consuming and not without risk. The goal is a safer and more prolonged method of delivery. This will come through the development of new therapies, the intrinsic chemical structure of which (low MW and high lipophilicity) will enable them to penetrate the eye more easily. This would enable less invasive delivery methods, such as topical administration, to be developed. In addition, the development of new technologies for sustained-release means that even if a drug has to be delivered via an intravitreal route, it need not be delivered very often.

Topical administration could be an ideal delivery method for the management of neovascular retinal disease, owing to a favourable side-effect profile and a reduced need for medical intervention. New topical agents in clinical testing are primarily smaller, lipophilic compounds, acting upon targets other than VEGF. Whether they will be a realistic alternative to anti-VEGF treatment is yet to be determined, as is the case for the challenges of compliance and accurate eye drop application in the elderly

and poorly sighted AMD population. VEGF blockade, although not without its limitations, has set a high standard of clinical effectiveness that new drugs may find difficult to beat. Unless a revolutionary new drug comes to market, VEGF blockade is likely to remain the bedrock of anti-angiogenic therapy. Being large proteins, anti-VEGF agents are best suited to intravitreal delivery. It is likely that the next shift in the treatment of retinal angiogenesis will be towards prolonged intravitreal delivery of current anti-VEGF agents, rather than the replacement of VEGF as the central target in the treatment of ocular angiogenesis.

References

1 Steinkuller, P.G. *et al.* (1999) Childhood blindness. *J. Am. Assoc. Pediatr. Ophthalmol. Strabismus* 3, 26–32
2 Rahmani, B. *et al.* (1996) The cause-specific prevalence of visual impairment in an urban population. The Baltimore Eye Survey. *Ophthalmology* 103, 1721–1726
3 Bressler, N.M. and Bressler, S.B. (1995) Preventative ophthalmology. Age-related macular degeneration. *Ophthalmology* 102, 1206–1211
4 Del Amo, E.M. and Urtti, A. (2008) Current and future ophthalmic drug delivery systems. A shift to the posterior segment. *Drug Discov. Today* 13, 135–143
5 Hori, Y. *et al.* (1996) Differential effects of angiostatic steroids and dexamethasone on angiogenesis and cytokine levels in rat sponge implants. *Br. J. Pharmacol.* 118, 1584–1591
6 Gragoudas, E.S. *et al.* (2004) Pegaptanib for neovascular age-related macular degeneration. *N. Engl. J. Med.* 351, 2805–2816
7 Kourlas, H. and Schiller, D.S. (2006) Pegaptanib sodium for the treatment of neovascular age-related macular degeneration: a review. *Clin. Ther.* 28, 36–44
8 Ruckman, J. *et al.* (1998) 2′-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. *J. Biol. Chem.* 273, 20556–20567
9 Rosenfeld, P.J. *et al.* (2006) Ranibizumab for neovascular age-related macular degeneration. *N. Engl. J. Med.* 355, 1419–1431
10 Brown, D.M. *et al.* (2006) Ranibizumab versus verteporfin for neovascular age-related macular degeneration. *N. Engl. J. Med.* 355, 1432–1444
11 The Royal College of Ophthalmologists (2008) Guidelines for Ranibizumab – the Clinician's Guide to commencing, continuing and discontinuing treatment (http://www.rcophth.ac.uk/about/publications)
12 Bakri, S.J. *et al.* (2007) Pharmacokinetics of intravitreal ranibizumab (Lucentis). *Ophthalmology* 114, 2179–2182
13 Riely, G.J. and Miller, V.A. (2007) Vascular endothelial growth factor Trap in non small cell lung cancer. *Clin. Cancer Res.* 13, s4623–s4627
14 Holash, J. *et al.* (2002) VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11393–11398
15 Nguyen, Q.D. *et al.* (2006) A phase I trial of an IV-administered vascular endothelial growth factor trap for treatment in patients with choroidal neovascularization due to age-related macular degeneration. *Ophthalmology* 113 1522.e1–1522.e14
16 Ambati, J. *et al.* (2000) Diffusion of high molecular weight compounds through sclera. *Invest. Ophthalmol. Vis. Sci.* 41, 1181–1185
17 Wang, W. *et al.* (1991) Lipophilicity influence on conjunctival drug penetration in the pigmented rabbit: a comparison with corneal penetration. *Curr. Eye Res.* 10, 571–579
18 Maurice, D. (2001) Review: practical issues in intravitreal drug delivery. *J. Ocul. Pharmacol. Ther.* 17, 393–401
19 Liu, W. *et al.* (1998) Pharmacokinetics of sparfloxacin in the serum and vitreous humor of rabbits: physicochemical properties that regulate penetration of quinolone antimicrobials. *Antimicrob. Agents Chemother.* 42, 1417–1423
20 la Cour, M. (2007) Intravitreal VEGF-inhibitors: is Avastin a generic substitute for Lucentis? *Acta Ophthalmol. Scand.* 85, 2–4
21 Presta, L.G. *et al.* (1997) Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res.* 57, 4593–4599
22 Stewart, M.W. and Rosenfeld, P.J. (2008) Predicted biological activity of intravitreal VEGF Trap. *Br. J. Ophthalmol.* 92, 667–668
23 Driot, J.Y. *et al.* (2004) Ocular pharmacokinetics of fluocinolone acetonide after Retisert intravitreal implantation in rabbits over a 1-year period. *J. Ocul. Pharmacol. Ther.* 20, 269–275
24 Bourges, J.L. *et al.* (2006) Intraocular implants for extended drug delivery: therapeutic applications. *Adv. Drug Deliv. Rev.* 58, 1182–1202
25 Pearson, P. *et al.* (2006) Fluocinolone acetonide intravitreal implant to treat diabetic macular edema: 3-year results of a multi-center clinical trial (Abstract). *Invest. Ophthalmol. Vis. Sci.* 47, 5442
26 Shane, T.S. and Martin, D.F. (2003) Endophthalmitis after ganciclovir implant in patients with AIDS and cytomegalovirus retinitis. *Am. J. Ophthalmol.* 136, 649–654
27 Dunn, J.P. *et al.* (2004) Complications of ganciclovir implant surgery in patients with cytomegalovirus retinitis: the Ganciclovir Cidofovir Cytomegalovirus Retinitis Trial. *Retina* 24, 41–50
28 Kimura, H. and Ogura, Y. (2006) Biodegradable systems. In *Intraocular Drug Delivery* (1st ed.) (Jaffe, G.J. *et al.* eds), pp. 175–192, Informa Healthcare
29 Kunou, N. *et al.* (2000) Long-term sustained release of ganciclovir from biodegradable scleral implant for the treatment of cytomegalovirus retinitis. *J. Control. Release* 68, 263–271
30 Hsu, J. (2007) Drug delivery methods for posterior segment disease. *Curr. Opin. Ophthalmol.* 18, 235–239
31 Moritera, T. *et al.* (1992) Biodegradable microspheres containing adriamycin in the treatment of proliferative vitreoretinopathy. *Invest. Ophthalmol. Vis. Sci.* 33, 3125–3130
32 Moshfeghi, A.A. and Peyman, G.A. (2005) Micro- and nanoparticulates. *Adv. Drug Deliv. Rev.* 57, 2047–2052
33 Lavinsky, D. *et al.* (2008) Phase I/II study of intravitreal triamcinolone acetonide microspheres for treatment of diffuse diabetic macular edema unresponsive to conventional laser photocoagulation treatment. *Invest. Ophthalmol. Vis. Sci.* 49 E-Abstract 2698 (online)
34 Li, H. *et al.* (2006) A PEDF N-terminal peptide protects the retina from ischemic injury when delivered in PLGA nanospheres. *Exp. Eye Res.* 83, 824–833
35 Schmidt, P.G. *et al.* (2007) PEGylated bioactive molecules in biodegradable polymer microparticles. *Expert Opin. Biol. Ther.* 7, 1427–1436
36 Vandervoort, J. and Ludwig, A. (2007) Ocular drug delivery: nanomedicine applications. *Nanomedicine* 2, 11–21
37 Doukas, J. *et al.* (2008) Topical administration of a multi-targeted kinase inhibitor suppresses choroidal neovascularization and retinal edema. *J. Cell. Physiol.* 216, 29–37
38 Lee, V.H. and Robinson, J.R. (1986) Topical ocular drug delivery: recent developments and future challenges. *J. Ocul. Pharmacol.* 2, 67–108
39 Prausnitz, M.R. and Noonan, J.S. (1998) Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye. *J. Pharm. Sci.* 87, 1479–1488
40 Hamalainen, K.M. *et al.* (1997) Characterization of paracellular and aqueous penetration routes in cornea, conjunctiva, and sclera. *Invest. Ophthalmol. Vis. Sci.* 38, 627–634
41 Fitzgerald, P. *et al.* (1987) A gamma scintigraphic evaluation of the precorneal residence of liposomal formulations in the rabbit. *J. Pharm. Pharmacol.* 39, 487–490
42 Kim, S.H. *et al.* (2007) Transport barriers in transscleral drug delivery for retinal diseases. *Ophthalmic Res.* 39, 244–254
43 Anderson, O.A. *et al.* (2008) Human transscleral albumin permeability and the effect of topographical location and donor age. *Invest. Ophthalmol. Vis. Sci.* 49, 4041–4045
44 Robinson, M.R. *et al.* (2006) A rabbit model for assessing the ocular barriers to the transscleral delivery of triamcinolone acetonide. *Exp. Eye Res.* 82, 479–487
45 Pontes de Carvalho, R.A. *et al.* (2006) Delivery from episcleral exoplants. *Invest. Ophthalmol. Vis. Sci.* 47, 4532–4539
46 Bill, A. (1964) The drainage of albumin from the uvea. *Exp. Eye Res.* 3, 179–187
47 Kim, H. *et al.* (2004) Controlled drug release from an ocular implant: an evaluation using dynamic three-dimensional magnetic resonance imaging. *Invest. Ophthalmol. Vis. Sci.* 45, 2722–2731
48 Kato, A. *et al.* (2004) Feasibility of drug delivery to the posterior pole of the rabbit eye with an episcleral implant. *Invest. Ophthalmol. Vis. Sci.* 45, 238–244
49 Okabe, J. *et al.* (2003) Biodegradable intrascleral implant for sustained intraocular delivery of betamethasone phosphate. *Invest. Ophthalmol. Vis. Sci.* 44, 740–744
50 Okabe, K. *et al.* (2003) Intraocular tissue distribution of betamethasone after intrascleral administration using a non-biodegradable sustained drug delivery device. *Invest. Ophthalmol. Vis. Sci.* 44, 2702–2707
51 Gilger, B.C. *et al.* (2006) A novel bioerodible deep scleral lamellar cyclosporine implant for uveitis. *Invest. Ophthalmol. Vis. Sci.* 47, 2596–2605
52 Olsen, T.W. *et al.* (2006) Cannulation of the suprachoroidal space: a novel drug delivery methodology to the posterior segment. *Am. J. Ophthalmol.* 142, 777–787

53 Bochot, A. *et al.* (1998) Comparison of the ocular distribution of a model oligonucleotide after topical instillation in rabbits of conventional and new dosage forms. *J. Drug Target.* 6, 309–313

54 Carrasquillo, K.G. *et al.* (2003) Controlled delivery of the anti-VEGF aptamer EYE001 with poly(lactic-co-glycolic)acid microspheres. *Invest. Ophthalmol. Vis. Sci.* 44, 290–299

55 Voigt, M. *et al.* (2002) Down-regulation of NOSII gene expression by iontophoresis of anti-sense oligonucleotide in endotoxin-induced uveitis. *Biochem. Biophys. Res. Commun.* 295, 336–341

56 Parkinson, T.M. *et al.* (2003) Tolerance of ocular iontophoresis in healthy volunteers. *J. Ocul. Pharmacol. Ther.* 19, 145–151

57 Halhal, M. *et al.* (2004) Iontophoresis: from the lab to the bed side. *Exp. Eye Res.* 78, 751–757

58 Eljarrat-Binstock, E. *et al.* (2008) Charged nanoparticles delivery to the eye using hydrogel iontophoresis. *J. Control. Release* 126, 156–161

59 Dufes, C. *et al.* (2005) Dendrimers in gene delivery. *Adv. Drug Deliv. Rev.* 57, 2177–2202

60 Bejjani, R.A. *et al.* (2005) Nanoparticles for gene delivery to retinal pigment epithelial cells. *Mol. Vis.* 11, 124–132

61 Bainbridge, J.W. *et al.* (2002) Inhibition of retinal neovascularisation by gene transfer of soluble VEGF receptor sFlt-1. *Gene Ther.* 9, 320–326

62 Pechan, P. *et al.* (2009) Novel anti-VEGF chimeric molecules delivered by AAV vectors for inhibition of retinal neovascularization. *Gene Ther.* 16, 10–16

63 Reichel, M.B. *et al.* (1998) Immune responses limit adenovirally mediated gene expression in the adult mouse eye. *Gene Ther.* 5, 1038–1046

64 Kuo, C.J. *et al.* (2001) Comparative evaluation of the antitumor activity of antiangiogenic proteins delivered by gene transfer. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4605–4610

65 Campochiaro, P.A. *et al.* (2006) Adenoviral vector-delivered pigment epithelium-derived factor for neovascular age-related macular degeneration: results of a phase I clinical trial. *Hum. Gene Ther.* 17, 167–176

66 Mori, K. *et al.* (2002) Regression of ocular neovascularization in response to increased expression of pigment epithelium-derived factor. *Invest. Ophthalmol. Vis. Sci.* 43, 2428–2434

67 Gehlbach, P. *et al.* (2003) Periocular injection of an adenoviral vector encoding pigment epithelium-derived factor inhibits choroidal neovascularization. *Gene Ther.* 10, 637–646

68 Saishin, Y. *et al.* (2005) Periocular gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization in a human-sized eye. *Hum. Gene Ther.* 16, 473–478

69 Bainbridge, J.W. *et al.* (2003) Gene therapy for ocular angiogenesis. *Clin. Sci. (Lond.)* 104, 561–575

70 Bainbridge, J.W. *et al.* (2008) Effect of gene therapy on visual function in Leber's congenital amaurosis. *N. Engl. J. Med.* 358, 2231–2239

71 Cideciyan, A.V. *et al.* (2008) Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15112–15117

72 Maguire, A.M. *et al.* (2008) Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N. Engl. J. Med.* 358, 2240–2248

73 McGee Sanftner, L.H. *et al.* (2001) Recombinant AAV-mediated delivery of a tet-inducible reporter gene to the rat retina. *Mol. Ther.* 3, 688–696

74 Auricchio, A. *et al.* (2002) Pharmacological regulation of protein expression from adeno-associated viral vectors in the eye. *Mol. Ther.* 6, 238–242

75 Bainbridge, J.W. *et al.* (2003) Hypoxia-regulated transgene expression in experimental retinal and choroidal neovascularization. *Gene Ther.* 10, 1049–1054

76 Sieving, P.A. *et al.* (2006) Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3896–3901

77 Uteza, Y. *et al.* (1999) Intravitreous transplantation of encapsulated fibroblasts secreting the human fibroblast growth factor 2 delays photoreceptor cell degeneration in Royal College of Surgeons rats. *Proc. Natl. Acad. Sci. U.S.A.* 96, 3126–3131

78 Hoane, M.R. *et al.* (2000) Mammalian-cell-produced neurturin (NTN) is more potent than purified Escherichia coli-produced NTN. *Exp. Neurol.* 162, 189–193