Drugs of the Future 2002, 27(9): 841-845

CCC: 0377-8282/2002

Pegaptanib Sodium

USAN

Treatment of Age-Related Macular Degeneration
Treatment of Diabetic Retinopathy
Anti-VEGF Aptamer

EYE-001
NX-1838
Macugen®

[(2'-Deoxy-2'-fluoro)C-G_m-G_m-A-A-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-A_m-G_m-(2'-deoxy-2'-fluoro)U-G_m-A_m-A_m-(2'-deoxy-2'-fluoro)U-G_m-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)U-A_m-(2'-deoxy-2'-fluoro)U-A_m-(2'-deoxy-2'-fluoro)C-A_m-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)C-G_m-(3'→3')-dT]RNA, 5'-ester with α,α'-[4,12-dioxo-6(*S*)-[[[5-(phosphonooxy)pentyl]amino]carbonyl]-3,13-dioxa-5,11-diaza-1,15-penta- decanediyl]bis-[ω-methoxypoly(oxy-1,2-ethanediyl)] octacosasodium salt

5'-ester of (2'-deoxy-2'-fluoro)C-G_m-G_m-A-A-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-A_m-G_m-(2'-deoxy-2'-fluoro)U-G_m-A_m-A_m-(2'-deoxy-2'-fluoro)U-G_m-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)U-A_m-(2'-deoxy-2'-fluoro)U-A_m-(2'-deoxy-2'-fluoro)C-A_m-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)C-G_m-(3'→3')-dT with α,α'-[[(1*S*)-1-[[5-(phosphonooxy)pentyl]carbamoyl]pentane-1,5-diyl]bis(iminocarbonyl)]bis[ω-methoxypoly-(oxyethane-1,2-diyl)] octacosasodium salt

CAS: 222716-86-1

EN: 264254

Abstract

Exudative age-related macular degeneration (AMD) and diabetic macular edema (DME) are the leading causes of vision loss in the elderly and diabetics, respectively, in the Western world. Although photocoagulation and photodynamic therapy are indicated for these pathologies, recurrence is exceptionally high. Thus, the search continues for a more effective treatment for these disorders. Vascular endothelial growth factor (VEGF) is a cytokine involved in angiogenesis and necessary for normal vascular development. However, it has also been implicated in several pathologies such as AMD, DME and choroidal neovascularization (CNV) where patients display high intraocular VEGF levels. Thus, anti-VEGF therapy is an attractive therapeutic option for these diseases. One such anti-VEGF agent is the pegylated aptamer pegaptanib sodium. Pegaptanib specifically binds with high affinity to $VEGF_{165}$, the major soluble human VEGF isoform, and has been shown to potently inhibit blood vessel growth and block neovascularization in preclinical models. It has been chosen for further development for the treatment of AMD and DME and is the first aptamer to reach human clinical testing.

Introduction

Although the most common cause of irreversible vision loss in the elderly in the Western world is exudative age-related macular degeneration (AMD) and diabetic macular edema (DME), there is no truly effective treatment available at the present time (1). Currently, thermal laser treatment or photocoagulation therapy is used although it is only effective in the small percentage of patients with well-defined, classic lesions, and even in these patients, there is a 50% rate of recurrence (2-4). Another option that is currently marketed in the U.S. is photodynamic therapy (PDT) with Visodyne™. However, it is only indicated for a small population of patients with classic choroidal neovascularization (CNV) who make up about one-third of all individuals with AMD. PDT has been shown to be effective although recurrence within 3 months of therapy is seen in more than 90% of the patients (5). It is clear that the search for other treatment options for AMD and DME is of paramount interest.

Vascular endothelial growth factor (VEGF) is a cytokine that plays a crucial role in angiogenesis (6, 7). There are four main isoforms, $VEGF_{206}$, $VEGF_{189}$, $VEGF_{165}$ and $VEGF_{121}$, of which only the first three can bind heparin via exon 7. The clinical significance of each

L.A. Sorbera, P.A. Leeson, M. Bayés. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

isoform is still unclear, although $VEGF_{165}$ appears to be the major isoform in humans. VEGF is known to be involved in normal vascular development and is required for neovascularization in the cornea, iris and retina. However, it has also been shown to be involved in several pathologies, including tumor growth where it is required for neovascularization (8-12). Research has shown that there are high concentrations of VEGF in the eyes of patients suffering from CNV, AMD or DME and it has been positively linked to ischemia-associated retinal neovascularization and exudative AMD (13-15). Suppression of VEGF activity may therefore represent an effective treatment option for management of diseases involving ocular neovascularization.

High-affinity antibodies directed to VEGF would be one method of abolishing VEGF activity. However, the currently available recombinant anti-VEGF antibodies are humanized proteins containing residual mouse sequences which can induce human antimouse immune responses. Another more interesting option would be development of an aptamer against VEGF. Aptamers are oligonucleotides specifically isolated to bind to specific molecular targets from randomized RNA, DNA or modified nucleic acid libraries using SELEX (systematic evolution of ligands by exponential enrichment) technology (16, 17). One such RNA-based aptamer developed for anti-VEGF therapy is the pegylated aptamer pegaptanib sodium (EYE-001, NX-1838). Pegaptanib sodium is the sodium salt of a 40 kD polyethylene glycol (PEG)-conjugated 28-mer oligonucleotide (18) that specifically binds with high affinity to $VEGF_{165}$, the major soluble human VEGF isoform. Pegaptanib can bind and inactivate VEGF in a manner similar to high affinity antibodies to VEGF. Pegaptanib has been shown to potently inhibit VEGF-induced vascular permeability, blood vessel growth and neovascularization in preclinical models and has been chosen for further development for the treatment of AMD and DME. It is the first aptamer to reach clinical testing.

Pharmacological Actions

Pegaptanib was shown to antagonize several VEGF-mediated cellular responses *in vitro*. The agent inhibited $VEGF_{165}$ binding to human umbilical vein endothelial cells (HUVECs) in addition to dose-dependently blocking $VEGF_{165}$-induced Ca^{2+} mobilization and proliferation. The estimated IC_{50} values for these effects ranged from 0.1-1 nM. $VEGF_{165}$-mediated phosphorylation of the tyrosine kinase receptor (KDR) and phospholipase Cγ (PLCγ) were also inhibited by the agent. The effects were similar to those observed with an anti-VEGF monoclonal antibody, although pegaptanib was unable to inhibit HUVEC proliferation induced by $VEGF_{121}$, a VEGF isoform that lacks exon 7 and cannot bind heparin; pegaptanib has been shown to have minimal binding affinity for $VEGF_{121}$. Furthermore, studies *in vivo* in guinea pigs (the Miles assay) showed that pegaptanib significantly decreased intradermal VEGF-induced vascular permeability by about 83% (18, 19).

Results from an *in vivo* study also using guinea pigs in the Miles assay in addition to a rat corneal angiogenesis model and a mouse retinopathy of prematurity model, demonstrated the potent antineovascularization effects of pegaptanib. In the Miles assay, the agent administrated intradermally at concentrations as low as 100 nM almost completely suppressed $VEGF_{165}$-mediated vascular leakage. Similarly, in the rat corneal angiogenesis model, pegaptanib (3 and 10 mg/kg once daily or b.i.d. i.v. for 5 days) significantly inhibited $VEGF_{165}$-dependent angiogenesis by 65% as compared to controls. Moreover, retinal neovascularization was significantly decreased by 80% in the mouse retinopathy of prematurity model with pegaptanib doses of 3 and 10 mg/kg once daily for 5 days. A dose of 1 mg/kg had little efficacy in these models (20).

Pegaptanib has also shown efficacy in inhibiting growth of human xenografts. Treatment of nude mice bearing established and nonestablished human A673 rhabdomyosarcoma xenografts with 10 mg/kg pegaptanib (i.p. once daily starting on day 1 postimplantation) inhibited growth of xenografts by 59 and 89%, respectively. Similar suppression in growth (69 and 83%, respectively) was seen in animals treated with an anti-VEGF monoclonal antibody (100 μg). Comparable data have been presented in studies showing inhibition of rhabdomyosarcoma xenograft growth ranging from 71% on day 6 of treatment to 76% on days 9 and 13 of pegaptanib treatment (10 mg/kg i.p. once daily) (20, 21).

A recent report has described the development of a controlled-drug delivery system for pegaptanib using poly(lactic-co-glycolic)acid microspheres. The microspheres delivered pegaptanib in a sustained manner averaging 2 μg/day for 20 days and the anti-VEGF efficacy of the agent was maintained. Experiments in which pegaptanib microspheres were packed into a sealed chamber and placed in the orbital part of rabbit sclera for 6 days confirmed delivery of the agent through the sclera. This delivery system therefore allows for long-term inhibition of VEGF-mediated effects with pegaptanib and may be effective in the transcleral treatment of choroidal and retinal diseases (22).

Pharmacokinetics

A high-performance liquid chromatographic anion-exchange method has been validated for determination of pegaptanib in plasma. Intact pegaptanib was measured with an accuracy ranging between 107-115% and a coefficient of variation of less than 8%. The method was tested in rhesus monkeys that received a single 1 mg/kg i.v. or s.c. dose. The maximum plasma concentration, terminal half-life and clearance rate following i.v. administration were 2.25 μg/ml, 9.3 h and 6.2 ml/h, respectively. The fraction of the dose absorbed into the plasma compartment after s.c. dosing was 0.78,

Box 1: Safety and efficacy of pegaptinib in the treatment of age-related macular degeneration (20) [Prous Science Integrity®].

Design	Open, multicenter, dose-escalating study
Population	Patients with subfoveal choroideal neovascularization secondary to exudative age-related macular degeneration (n=15)
Treatments	Pegaptanib, 0.25 mg intravitreal s.d. Pegaptanib, 0.5 mg intravitreal s.d. Pegaptanib, 1 mg intravitreal s.d. Pegaptanib, 2 mg intravitreal s.d. Pegaptanib, 3 mg intravitreal s.d.
Adverse Events	Mild intraocular inflammation, scotoma, visual distortion, hives, eye pain, fatigue Breast carcinoma (1/15, 6.7%) [not drug-related]
Conclusions	Pegaptanib appeared to be safe and effective in improving or stabilizing vision at 3 months in 80% of patients with subfoveal choroideal neovascularization secondary to exudative age-related macular degeneration

Box 2: Efficacy and safety of pegaptanib in the treatment of age-related macular degeneration (25) [Prous Science Integrity®].

Design	Open
Population	Patients with subfoveal choroideal neovascularization secondary to exudative age-related macular degeneration (n=36)
Treatments	Pegaptanib, intravitreal s.d.
Conclusions	Pegaptanib appeared to be safe and effective in improving or stabilizing vision at 3 months in 80% of patients with subfoveal choroideal neovascularization secondary to exudative age-related macular degeneration

with peak concentrations of 4.9 μg/ml achieved at 8-12 h (23).

Another study conducted using rhesus monkeys reported both the safety and plasma and vitreous pharmacokinetics of single intravitreal bilateral doses of pegaptanib (0.25, 0.50, 1, 1.5 or 2 mg/eye). Both plasma and vitreous pharmacokinetics were linearly related to dose. The agent was cleared from the vitreous humor into plasma with a half-life value of about 94 h for all doses. Pegaptanib found in the vitreous humor after 28 h was shown in VEGF-binding assays to be fully active. Plasma half-life values following intravitreal injection of 0.5, 1.5 and 2 mg/eye were 102.2 ± 21.5, 87.4 ± 0.31 and 88.9 ± 10.5 h, respectively. Multiple dosing (6 biweekly bilateral intravitreal injections) with pegaptanib proved to be safe with no toxicological effects or antibody responses noted (24).

The pharmacokinetics of intravitreal pegaptanib have also been reported in rabbits. Initial vitreous humor concentrations of the agent following a single bilateral dose (0.35 mg/eye) were approximately 350 μg/ml, later decreasing by a first-order elimination process to about 1.7 μg/ml by day 28 postinjection. Similar to rhesus monkeys, the elimination half-life was 83 h. Vitreous humor pegaptanib concentrations were about 190 nM, above the K_d for VEGF (200 pM), even at 4 weeks postdosing. Plasma concentrations of the agent were lower than those found in the vitreous humor, ranging from 0.092 to 0.005 μg/ml from day 1 to day 21. The plasma terminal half-life was estimated to be 84 h with elimination following a first-order process. Thus, intravitreal pegaptanib is highly stable and slowly released from the vitreous humor into the systemic circulation (20).

Clinical Studies

Results from a multicenter, open-label, dose-escalation, phase IA study involving 15 patients (64-92 years old) with subfoveal CNV secondary to exudative AMD showed the safety and efficacy of a single intravitreal injection of pegaptanib (0.25, 0.5, 1, 2 and 3 mg/eye). The dose-limiting toxicity was not reached in this study and viscosity of formulations over 3 mg prevented further dose escalation. A total of 17 mild or moderate adverse events were experienced by 11 patients of which 6 (mild intraocular inflammation, scotoma, visual distortion, hives, eye pain and fatigue) were probably or possibly drug-related; 1 serious adverse event (breast carcinoma) was noted but was unrelated to treatment. At 3 months postinjection, 80% of the patients had stable or improved vision and 26.7% had significantly improved vision (an increase of 3 or more lines on the Early Treatment for Diabetic Retinopathy Study [ETDRS] chart). No signs of retinal or choroidal toxicity were observed with treatment (20) (Box 1).

Results have been presented from phase I and II trials showing the efficacy and safety of intravitreal pegaptanib. A study was conducted in 36 patients with

CNV who received intravitreal injections of the agent. At 3 months postdosing, 80% of the patients had stable or improved vision and about 30% had an improvement of 3 or more lines on the ETDRS chart. No serious adverse events related to pegaptanib were reported. Preliminary results from patients treated with a combination of pegaptanib and PDT indicate a better improvement in vision with no serious adverse advents (25) (Box 2).

Pegaptanib sodium continues to undergo phase II/III clinical studies for the treatment of AMD and DME. Eyetech has completed enrollment of a phase II/III multicenter, randomized, double-blind, controlled, comparative trial to examine the safety and efficacy of intravitreal pegaptanib injection alone or in combination with PDT in patients with exudative AMD. Enrollment for a trial investigating the efficacy of pegaptanib as a treatment for DME is scheduled (26).

Source

Discovered by Gilead Sciences, Inc. (US); licensed worldwide to EyeTech Pharmaceuticals, Inc. (US).

References

1. Hyman, L. *Epidemiology of eye disease in the elderly.* Eye 1987, 1: 330-41.

2. Macular Photocoagulation Study Group. *Krypton laser photocoagulation for neovascular lesions of age-related macular degeneration. Results of a randomized clinical trial.* Arch Ophthalmol 1990, 108: 816-24.

3. Macular Photocoagulation Study Group. *Argon laser photocoagulation for neovascular maculopathy. Five-year results from randomized clinical trials.* Arch Ophthalmol 1991, 109: 1109-14.

4. Macular Photocoagulation Study Group. *Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial.* Arch Ophthalmol 1991, 109: 1220-31.

5. Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) Study Group. *Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: One-year results of 2 randomized clinical trials - TAP report.* Arch Ophthalmol 1999, 117: 1329-45.

6. Ferrara, N., Houck, K., Jakeman, L., Leung, D.W. *Molecular and biological properties of the vascular endothelial growth factor family of proteins.* Endocr Rev 1992, 13: 18-32.

7. Leung, D.W., Cachianes, G., Kuang, W.J., Goeddel, D.V., Ferrara, N. *Vascular endothelial growth factor is a secreted angiogenic mitogen.* Science 1989, 246: 1306-9.

8. Carmeliet, P., Ferreira, V., Breier, G. et al. *Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele.* Nature 1996, 380: 435-9.

9. Kim, K.J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H.S., Ferrara, N. *Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo.* Nature 1993, 362: 841-4.

10. Amano, S., Rohan, R., Kuroki, M., Tolentino, M., Adamis, A.P. *Requirement for vascular endothelial growth factor in wound- and inflammation-related corneal neovascularization.* Invest Ophthalmol Vis Sci 1998, 39: 18-22.

11. Adamis, A.P., Shima, D.T., Tolentino, M.J., Gragoudas, E.S., Ferrara, N., Folkman, J., D'Amore, P.A., Miller, J.W. *Inhibition of vascular endothelial growth factor prevents retinal ischemia-associated iris neovascularization in a nonhuman primate.* Arch Ophthalmol 1996, 114: 66-71.

12. Aiello, L.P., Pierce, E.A., Foley, E.D., Takagi, H., Chen, H., Riddle, L., Ferrara, N., King, G.L., Smith, L.E. *Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins.* Proc Natl Acad Sci USA 1995, 92: 10457-61.

13. Adamis, A.P., Miller, J.W., Bernal, M.T., D'Amico, D.J., Folkman, J., Yeo, T.K., Yeo, K.T. *Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy.* Am J Ophthalmol 1994, 118: 445-50.

14. Aiello, L.P., Avery, R.L., Arrigg, P.G. et al. *Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders.* New Engl J Med 1994, 331: 1480-7.

15. Smith, L.E., Wesolowski, E., McLellan, A., Kostyk, S.K., D'Amato, R., Sullivan, R., D'Amore, P.A. *Oxygen-induced retinopathy in the mouse.* Invest Ophthalmol Vis Sci 1994, 35: 101-11.

16. Tuerk, C., Gold, L. *Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase.* Science 1990, 249: 505-10.

17. Ellington, A.D., Szostak, J.W. *In vitro selection of RNA molecules that bind specific ligands.* Nature 1990, 346: 818-22.

18. Ruckman, J., Green, L.S., Beeson, J., Waugh, S., Gillette, W.L., Henninger, D.D., Claesson-Welsh, L., Janjic, N. *2'-Fluoropyrimidine RNA-based aptamers to the 165 amino acid form of vascular endothelial growth factor ($VEGF_{165}$). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain.* J Biol Chem 1998, 273: 20556-67.

19. Bell, C., Lynam, E., Landfair, D.J., Janjic, N., Wiles, M.E. *Oligonucleotide NX1838 inhibits $VEGF_{165}$-mediated cellular responses in vitro.* In Vitro Cell Dev Biol Anim 1999, 35: 533-42.

20. The Eyetech Study Group. *Preclinical and phase 1A clinical evaluation of an anti-VEGF pegylated aptamer (EYE001) for the treatment of exudative age-related macular degeneration.* Retina - J Retin Vitr Dis 2002, 22: 143-52.

21. Tomkinson, B., Bendele, R., Bill, J., Bridonneau, P., Brown, E., Janjic, N., LeRay, S.G.J., Emerson, D. *NX1838, a VEGF-specific antagonist aptamer, inhibits the growth of human xenografts in nude mice and enhances a suboptimal dose of Taxol.* Proc Am Assoc Cancer Res 1999, 40: Abst 4097.

22. Carrasquillo, K.G., Ricker, J., Rigas, I.K., Adamis, A.P. *Controlled delivery of anti-VEGF aptamer EYE001 with poly(lactic-co-glycolic)acid.* Annu Meet Assoc Res Vision Ophthalmol (May 5-10, Fort Lauderdale) 2002, Abst 2324.

23. Tucker, C.E., Chen, L.-S., Judkins, M.B., Farmer, J.A., Gill, S.C., Drolet, D.W. *Detection and plasma pharmacokinetics of an anti-vascular endothelial growth factor oligonucleotide-aptamer (NX1838) in rhesus monkeys.* J Chromatogr B - Biomed Sci Appl 1999, 732: 203-12.

24. Drolet, D.W., Nelson, J., Tucker, C.E. et al. *Pharmacokinetics and safety of an anti-vascular endothelial growth factor aptamer (NX1838) following injection into the vitreous humor of rhesus monkeys.* Pharm Res 2000, 17: 1503-10.

25. Mones, J. *Anti-VEGF therapy for patients with exudative age-related macular degeneration: Results of phase I and II clinical trials.* 4th Int Symp Ocular Pharmacol Pharm (Feb 28- March 3, Seville) 2002, 27.

26. *EyeTech completes enrollment in two pivotal Macugen trials.* DailyDrugNews.com (Daily Essentials) August 12, 2002.