



OPEN ACCESS

Ziv-aflibercept in macular disease

Ahmad M Mansour,¹ Sara I Al-Ghadban,² Muhammad H Yunis,¹
Marwan E El-Sabban²

¹Departments of Ophthalmology, American University of Beirut, and Rafic Hariri University Hospital, Beirut, Lebanon

²Department of Anatomy, Cell Biology and Physiological Sciences, American University of Beirut, Beirut, Lebanon

Correspondence to

Dr Ahmad Mansour, Department of Ophthalmology, American University of Beirut, Beirut 1136044, Lebanon; ammansourmd@gmail.com

Received 26 October 2014

Revised 5 January 2015

Accepted 12 January 2015

Published Online First

12 February 2015

ABSTRACT

Background/aims Aflibercept is an approved therapy for neovascular age-related macular degeneration (AMD) and diabetic macular oedema (DME). In vitro and in vivo studies did not detect toxicity to the retinal pigment epithelium cells using the approved cancer protein, ziv-aflibercept. Our purpose is to determine if ziv-aflibercept can be used in AMD and DME without ocular toxicity, to test the stability of ziv-aflibercept, and to do a cost analysis.

Methods Prospectively, consecutive patients with AMD or DME and poor vision underwent one intravitreal injection of 0.05 mL of fresh filtered ziv-aflibercept (1.25 mg). Monitoring of best-corrected visual acuity, intraocular inflammation, cataract progression, and retinal structure by spectral domain optical coherence tomography was done at 1 day and 1 week after injection. Ziv-aflibercept activity over 4 weeks was measured by capturing vascular endothelial growth factor by ELISA.

Results There were no signs of retinal toxicity, intraocular inflammation or change in lens status in four eyes with AMD and two eyes with DME. Visual acuity improved ($p=0.05$) and central foveal thickness decreased in all patients ($p=0.05$). Ziv-aflibercept had no loss of anti-VEGF activity when kept at 4°C in polycarbonate syringes over 4 weeks. Similar to bevacizumab, compounded ziv-aflibercept would yield a tremendous saving compared with aflibercept or ranibizumab.

Conclusions Off-label use of ziv-aflibercept improves visual acuity without ocular toxicity and may offer a cheaper alternative to the same molecule aflibercept.

Trial registration number NCT02173873.

INTRODUCTION

Aflibercept (Eylea; Regeneron, Tarrytown, New York, USA and Bayer Healthcare, Leverkusen, Germany) is a fusion protein consisting of the Fc portion of human immunoglobulin IgG1 and the extracellular domains of vascular endothelial growth factor receptors (VEGFR-2 and VEGFR-1), which binds to circulating vascular endothelial growth factor (VEGF), thus acting as a decoy receptor. Laboratory studies and clinical trials suggest that aflibercept's high binding affinity for VEGF may impart greater durability of activity and similar efficacy compared with ranibizumab¹ or bevacizumab. Aflibercept is approved by the US Food and Drug Administration (FDA) for the treatment of wet age-related macular degeneration (AMD),² macular oedema from retinal vein occlusion or diabetes.³ Ranibizumab is given monthly, while aflibercept is given bimonthly after 3 monthly injections for eyes with wet AMD. Because of the high cost of ranibizumab and aflibercept, a majority of ophthalmologists worldwide tend to treat

patients with bevacizumab^{4 5} at a major saving for the patient. Commercially, a much cheaper yet identical fusion protein to aflibercept is ziv-aflibercept. Ziv-aflibercept (Zaltrap, Sanofi-Aventis US, LLC, Bridgewater, New Jersey, USA and Regeneron Pharmaceuticals, Inc, Tarrytown, New York, USA) was approved by the FDA in August 2012 for the treatment of metastatic colorectal carcinoma resistant to an oxiplatin-containing regimen. One may wonder if ziv-aflibercept can be used instead of aflibercept in certain retinal disorders. Hence the need to answer some major safety concerns: first the difference in osmolarity, and second whether ziv-aflibercept could impair retinal function and alter morphology.⁶ A preliminary study was conducted on the use of ziv-aflibercept in patients with exudative AMD or diabetic macular oedema (DME) with poor vision. In addition, we tested the stability of ziv-aflibercept over a period of 4 weeks and the economic implications of the use of compounded drug.

METHODS

Ziv-aflibercept is supplied in single-use vials of 100 mg per 4 mL and 200 mg per 8 mL formulated as 25 mg/mL ziv-aflibercept in polysorbate 20 (0.1%), sodium chloride (100 mM), sodium citrate (5 mM), sodium phosphate (5 mM) and sucrose (20%), in Water for Injection USP, at a pH of 6.2. Eylea is supplied as a single-use, glass vial designed to deliver 0.05 mL (2 mg) of aflibercept (40 mg/mL in 10 mM sodium phosphate, 40 mM sodium chloride, 0.03% polysorbate 20, and 5% sucrose, pH 6.2). We injected 0.05 mL (1.25 mg) of ziv-aflibercept prepared fresh (within hours) from a new 4 mL vial. This was done under the hood in a sterile way using 1 mL BD syringe with Luer-Lok tip, clear polycarbonate barrel (Becton Dickinson and Company, Sparks, Maryland, USA) and filter needle. The fresh vial was punctured once and the drug was used within hours after preparation after storing it in the refrigerator at 4°C. After instillation of topical anaesthesia and povidone iodine solution, a sterile eyelid retractor was placed. The medication was injected 3.5 mm from the limbus into the mid-vitreous cavity. Best corrected visual acuities were monitored 15 min, 1 day and 1 week after injection by the same examiner using Snellen charts. Optical coherence tomography (OCT) was performed before the injection and after 1 week to look for possible side effects of the medication using high-resolution scans from RTVue-100 (software V.6.7, Optovue, Inc, Fremont, California, USA). Central macular thickness (CMT) was determined on the same machine and by the same independent operator. The height of the retinal pigment epithelium detachment was measured on the horizontal scan and vertical scan passing



Open Access
Scan to access more
free content



CrossMark

To cite: Mansour AM, Al-Ghadban SI, Yunis MH, et al. *Br J Ophthalmol* 2015;**99**:1055–1059.

through the foveola by averaging the horizontal and vertical measures. Inclusion criteria included eyes with active neovascular AMD or DME, best-corrected visual acuity of 20/100 (6/30) or less, ability to understand the risks and benefits of the study and ability to sign the formal consent form. Exclusion criteria included signs of ocular infection, prior periocular or intraocular corticosteroid usage, prior anti-VEGF therapy in the past 3 months, and history of cerebrovascular accident or myocardial infarct. Statistical analyses were done using the non-parametric Wilcoxon Signed-Ranks test. The research protocol received approval by the Institutional Review Board in May 2014. The study was registered (NCT02173873 <http://www.clinicaltrials.gov>). Clinical data obtained were analysed using SPSS V20.0 (IBM/SPSS Inc, Chicago, Illinois, USA). Paired sample t test was used to analyse the difference between the 1-week outcome and baseline values for visual acuity and CMT.

In vitro ELISA testing of VEGF binding

Ziv-aflibercept was drawn from a new vial using 19-gauge 5 µ BD filter into 1 mL BD Luer-Lok syringe with polycarbonate barrel (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) syringes and refrigerated at 4°C for 2 and 4 weeks. A fresh vial of ziv-aflibercept was used as control and reference. We used recombinant human VEGF165 (rhVEGF) (cat no. 293-VE-010, R&D Systems, Minneapolis, Minnesota, USA) and human VEGF Quantikine ELISA Kit (cat no. DVE00, R&D Systems). Different concentrations of ziv-aflibercept samples (10–10 mg/mL) stored were co-incubated with rhVEGF (100 ng/mL) diluted in phosphate-buffered saline. The ziv-aflibercept-rhVEGF complex (200 µL per well) was incubated for 30 min at room temperature and assayed for residual rhVEGF. The ELISA plate was incubated for 3 h at room temperature with agitation. The plate was then washed with phosphate-buffered saline to remove unbound complex. An antibody for VEGF (biotin-conjugated) was then added to the wells. Following a wash to remove unbound antibody, a detection reagent (streptavidin-hydrogen peroxidase) was added to bind the biotin-labelled detection antibody. The plate was washed and a substrate solution (3,3',5,5'-tetramethylbenzidine/hydrogen peroxide) was then added. Finally, a stop solution (sulfuric acid) was added and the optical densities (A₄₅₀ and A₅₅₀) were read using a spectrophotometer.

RESULTS

Four consecutive patients with wet AMD and two consecutive patients with DME received a single injection of intravitreal ziv-aflibercept in one eye (table 1).

These patients could not afford initial or additional injections of anti-VEGF agents and were actively seeking compassionate diagnostic and therapeutic regimens. Visual acuity improved on the first postoperative day in all patients with no sign of inflammation in the anterior chamber and vitreous, absence of retinal detachment or retinal haemorrhage. There was no progression of cataract or posterior capsular opacification. Intraocular pressure was not monitored. We checked only the vision 10 min after the injection and it was equal to the preinjection level in all cases. In four patients with AMD, the height of the foveolar detachment of the retinal pigment epithelium decreased in four patients from a mean of 583 µ to a final mean of 398 µ 1 week after injection. Combining the outcome for the six cases, the mean (SD) logMAR initial visual acuity was 1.40 (0.36) and at 1 week 0.86 (0.17) ($p=0.05$). Similarly, the mean initial CMT was 482 µ (217 µ) and decreased at 1 week to 345 µ (111 µ) ($p=0.05$).

The original vial concentration of ziv-aflibercept was 25 mg/mL (and in the current study) and this coincided with the known therapeutic dose (10–40 mg/mL) for aflibercept.² For that reason, we chose the highest concentration at 10 mg/mL. The stability of ziv-aflibercept was assessed by its efficiency to capture rhVEGF by measuring the concentration of free VEGF not bound to complex. At 10 mg/mL, ziv-aflibercept bound 90% of rhVEGF and this binding was stable over the entire test period (4 weeks). However, this binding was gradually decreased at lower concentrations of ziv-aflibercept. At 10 µg/mL, ziv-aflibercept bound 60% to rhVEGF and this binding decreased to 45% and 25% after 2 and 4 weeks, respectively (table 2).

Using this low concentration, we demonstrated the ability of ziv-aflibercept to capture free VEGF in clinical settings. Ziv-aflibercept was stable for 4 weeks at 4°C at a concentration higher than 100 µg/mL (knowing that the concentration of the drug at the time of injection is expected to be around 1.25 mg/4 mL of vitreous or 300 µg/mL).

The projected compounded cost is 20 times less for ziv-aflibercept than for aflibercept, if the 4 mL ziv-aflibercept vial is divided into 40 aliquots (table 3).

Table 1 Clinical profile of the ziv-aflibercept study

Patient number	Age	Race gender	Initial vision	Final vision	Initial CMT in micron	CMT 1 week after injection	Height of RPE detachment (initial to final, µ)	Subretinal fluid	Prior therapies	Systemic disease
Case 1 (AMD)	80	Caucasian Male	20/800	20/150	331	255	833–500	Yes	No	No
Case 2 (AMD)	89	Caucasian Female	20/100	20/60	748	473	1040–833	Yes (minimal)	11 bevacizumab/ 1 ranibizumab over 7.5 years	HTN; CAD
Case 3 (AMD)	84	Caucasian Male	20/800	20/200	213	181	271–90	Yes (minimal)	5 bevacizumab over 14 months	DM; CAD; smoker
Case 4 (AMD)	86	Caucasian Male	20/400	20/200	372	347	189–170	Yes (minimal)	4 bevacizumab over 14 months	HTN
Case 5 (diabetic maculopathy)	69	Caucasian Male	20/800	20/100	508	443	None	Yes	None	DM
Case 6 (diabetic maculopathy)	61	Caucasian Male	20/800	20/200	720	368	None	Yes (minimal)	Ranibizumab 2 Bevacizumab 1	DM

AMD, age-related macular degeneration; CAD, coronary artery disease; CMT, central macular thickness; DM, diabetes mellitus; HTN, systemic hypertension.

Table 2 Vascular endothelial growth factor (VEGF) binding capacity to ziv-aflibercept over a period of 28 days

Samples	Time points (week)		
	0 week (%)	2 weeks (%)	4 weeks (%)
VEGF-ziv-aflibercept 10 mg/mL	90±3.5	90±2	90±3
VEGF-ziv-aflibercept 1 mg/mL	80±4	80	80±4
VEGF-ziv-aflibercept 100 µg/mL	60±3	58	60±2
VEGF-ziv-aflibercept 10 µg/mL	62±2	45±1	24±1

This can be achieved in two ways: direct puncture of the vial for each patient as recommended by Ng *et al*,⁷ Chen *et al*⁸ and Ornek *et al*,⁹ or division by a compounding pharmacy in view of the excellent stability profile of the drug. The reason for the economic discrepancy after 1 year of therapy is twofold. First, use of divided or compounded ziv-aflibercept; second, a less intensive dosing regimen with ziv-aflibercept (8 doses for ziv-aflibercept or aflibercept vs 12 for ranibizumab or bevacizumab) (table 3).

DISCUSSION

Aflibercept has a molecular weight of 115 kDa and is manufactured from Chinese hamster ovary cells. Aflibercept is identical in structure to the cancer drug, ziv-aflibercept; however, it undergoes a different purification process and contains different buffer solutions that are less irritating when injected intravitreally.⁶ Since ziv-aflibercept and aflibercept have an identical molecular structure, we decided to test ziv-aflibercept (0.125 mg) which has a reduced concentration compared with aflibercept (2 mg). Aflibercept comes as an iso-osmotic solution (300 mOsm/kg), while ziv-aflibercept has an osmolarity of 1000 mOsm/kg. Marmor *et al*¹⁰ showed in rabbits and primates that solutions of less than 500 mOsm caused no retinal pigment epithelium damage. Tonicity between 500 and 1000 mOsm produced inconsistent and often only partial damage. Injections of more than 2000 mOsm promptly produced severe changes in

the retinal pigment epithelium with loss of villi and retinal detachment. The rabbit eye volume is on average around 1.5 mL, the primate eye volume ranges from 1.5 to 2.0 mL, while the human eye volume ranges from 4.0 to 6.0 mL. The volume injected in these experiments was 0.05 mL. Because the volume of the human eye is around three times larger than in the rabbit, it is expected that human eyes would theoretically tolerate more hyperosmolar injections than rabbit eyes, hence the higher threshold for osmolarity in humans. The absence of signs of toxicity in the 1000 mOsm/kg ziv-aflibercept human injection group relates to the small volume of the drug injected that did not result in a major shift in the total osmolarity of the vitreous. Diluting 0.05 mL of the drug into 4 mL of vitreous represents 80 times dilution so 1000 mOsm/kg solution will be diluted after injection in the vitreous (original 300 mOsm/kg to a calculated final osmolarity of 312 mOsm/kg or a 4% increase; ie, within the physiological range) and does not affect the retina.^{11 12} Ziv-aflibercept needs to be injected into the mid-vitreous cavity and away from the lens as a precautionary measure.

Laboratory studies have demonstrated the relative safety of ziv-aflibercept in animal eyes and cell preparation. In a study by Malik *et al*,¹³ human retinal pigment epithelium cells were exposed for 24 h to four anti-VEGF drugs at 1/2 times, 1 times, 2 times and 10 times clinical concentrations. Cell viability and mitochondrial membrane potential assay were performed to evaluate early apoptotic changes and rate of overall cell death. At clinical doses, neither ranibizumab nor aflibercept produced evidence of mitochondrial toxicity or cell death. However, bevacizumab and ziv-aflibercept showed mild mitochondrial toxicity at clinically relevant doses. In another study by Klettner *et al*,¹⁴ aflibercept displayed no cytotoxicity on retinal pigment epithelium cells while it impaired the phagocytic capacity of these cells. Recently data emerged showing that ziv-aflibercept was safe in rabbit eyes¹⁵: 18 rabbits were given intravitreal injection of 0.05 mL ziv-aflibercept or aflibercept. All eyes were negative for cataract and retinal detachment 1 and 7 days after injection without anatomic signs of toxicity by OCT and histology.

Table 3 Comparative anti-vascular endothelial growth factor (anti-VEGF) medications with the approved protocols and costs of the related drug therapies in the first year of therapy for wet AMD

Drug (company)	Ranibizumab (Genentech; Novartis)	Bevacizumab (Genentech; Roche)	Aflibercept (Regeneron; Bayer)	Ziv-aflibercept (Bayer)
Molecular weight	48 kDa	149 kDa	115 kDa	115 kDa
Half-life (serum) ²³	4.75 days	8.25 days	18 days	18 days
Half-life(vitreous)	2.75 days ²⁴	4.9 days ²⁵	7.1 days ²³	7.1 days ²³
Affinity to VEGF165 ²³	Kd 46 Pm*	Kd 58 Pm	Kd 0.49 Pm	Kd 0.49 Pm
Inhibit	VEGF-A	VEGF-A	VEGF-A; VEGF-B; PlGF	VEGF-A; VEGF-B; PlGF
Vial cost	£741 †	£242.7‡	£816§	£295.7¶
Cost per eye	£741	£40†	£816	£48
Cost per year 1	£8892	£480	£6528	£384
Vial dose and volume	2.3 mg in 0.23 mL	100 mg in 4 mL	4 mg in 0.1 mL	100 mg in 4 mL
AMD regimen	0.5 mg monthly	1.25 mg monthly	2 mg monthly 3 times then bimonthly	1.25 mg monthly 3 times then bimonthly
Injection volume	0.05 mL	0.05 mL	0.05 mL	0.05 mL
No. injections in year 1	12	12	8	8

Costs (in £=English pound) according to the British National Formulary edition 66.

*Affinity expressed as equilibrium dissociation constant (KD) in the picomolar (Pm) range.

†http://www.pmlive.com/pharma_news/novartis_nhs_price_lucentis_wet_amd_roche_avastin_402440 (accessed Jul 2014).

‡National Institute for Health and Care Excellence (NICE) technology appraisal guidance 242 (<http://www.nice.org.uk/guidance/ta242/chapter/the-technologies>) (accessed Jul 2014).

§NICE technology appraisal guidance 305 issued February 2014 (<http://guidance.nice.org.uk/ta305>). ¶NICE technology appraisal guidance 617 issued March 2014 (<http://guidance.nice.org.uk/TA/Wave0/617>).

AMD, age-related macular degeneration; PlGF, placental growth factor.

In VIEW 1 and 2, patients were randomised to one of four groups²: aflibercept 2 mg every 8 weeks (after three initial monthly doses), aflibercept 2 mg every 4 weeks, aflibercept 0.5 mg every 4 weeks and ranibizumab 0.5 mg every 4 weeks. The four groups had comparable primary endpoint, that is, the proportion of patients maintaining vision (defined as losing <15 letters on an ETDRS chart) at 52 weeks. In the current pilot study, the dose used was 1.25 mg of ziv-aflibercept (halfway between the 2 mg and 0.5 mg doses studied for aflibercept²). Hence the current dose for ziv-aflibercept falls within the proven therapeutic doses of aflibercept. An alternative solution is to inject 0.08 mL of ziv-aflibercept to deliver a total of 2 mg of the drug.

Currently, bevacizumab is the most cost-effective medication^{16–18} for control of wet AMD compared with ranibizumab (40 times cheaper⁵) or aflibercept. Yannuzzi *et al*¹⁹ performed a prospective in vitro study of syringes containing intravitreal preparations of bevacizumab from several compounding pharmacies in the USA. Similar findings were previously reported in pharmacies in France,²⁰ Italy²¹ and the UK.²² There were no microbial contaminants or endotoxin detected in any of the samples. Either sharing multiple doses of ziv-aflibercept from a single vial^{7–9} or using compounded ziv-aflibercept can substantially reduce the cost of treatment and with a softer regimen of 8 injections instead of 12 injections in the first year, ziv-aflibercept would appear theoretically to be more cost effective than bevacizumab (table 3),^{23–25} with unique features of high binding affinity, long half-life, and binding tightly to three isoforms of growth factors VEGF-A, VEGF-B and placental growth factor.²³ Unlike aflibercept, ziv-aflibercept is formulated in hypertonic sucrose, a condition that theoretically prevents its intraocular use because hypertonic preparations could damage the retina. According to Silver,⁵ the manufacturing differences between intraocular aflibercept and intravenous ziv-aflibercept are proprietary but unlikely to account for the 100-fold price differential.

The limitations of the current study include the small number of eyes treated, potential selection bias, and short duration of follow-up in these treated patients. It is possible to have cumulative toxic damage with ziv-aflibercept with multiple injections unlike with single injections. Additional concerns relate to previous reports of aflibercept-related intraocular inflammation²⁶ and it is likely that the use of ziv-aflibercept would be accompanied by sterile endophthalmitis when used in a large scale or used repeatedly. The current pilot study was not aimed at demonstrating significant visual improvement or flattening of the macula after injection but aimed at proving the concept that ziv-aflibercept is a potential substitute for aflibercept, especially in underprivileged countries with a very low national income, in the same way as bevacizumab is an economic substitute for ranibizumab. Ziv-aflibercept at concentrations even lower than used clinically was stable for 4 weeks at 4°C at a concentration higher than 100 µg/mL. This study was carried out in polycarbonate barrel syringes, with the knowledge that polycarbonate syringes have poor binding to protein and an excellent safety profile.²⁷ Future clinical studies can reaffirm the long-term safety and efficacy of ziv-aflibercept in various retinal diseases. In conclusion, in the current six cases, visual loss, retinal detachment and intraocular inflammation were not noted after intravitreal injection of ziv-aflibercept. Ziv-aflibercept deserves further investigation in clinical trials as an alternative cost-effective therapy for retinal diseases requiring anti-VEGF therapy and is a very attractive alternative to bevacizumab, ranibizumab and aflibercept due to its lower cost and long durability of action. It could also provide a second line of therapy in eyes with wet AMD

or DME resistant to bevacizumab therapy in underprivileged countries.

Acknowledgements Professor Abdul Razzak Charbaji, PhD, Department of Statistics and Research Methodology, Lebanese American University and Lebanese University, Beirut, Lebanon for statistical analysis.

Contributors Design (AMM, MEE-S) and conduct (AMM, MHY, MEE-S, SIA-G) of the study; collection (AMM, MHY), management (AMM, MHY, MEE-S, SIA-G), analysis (AMM, MEE-S, SIA-G), and interpretation of the data (AMM, MEE-S, SIA-G); and preparation (AMM, SIA-G), review (AMM, MEE-S, SIA-G) and approval (AMM, MHY, MEE-S, SIA-G) of the manuscript.

Competing interests Ahmad Mansour is a consultant for Bayer, Leverkusen, Germany.

Patient consent Obtained.

Ethics approval IRB RHUH.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All original data are available from Ahmad Mansour.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

REFERENCES

- Schmid MK, Bachmann LM, Fäs L, *et al*. Efficacy and adverse events of aflibercept, ranibizumab and bevacizumab in age-related macular degeneration: a trade-off analysis. *Br J Ophthalmol* 2015;99:141–6.
- Schmidt-Erfurth U, Kaiser PK, Korobelnik JF, *et al*. Intravitreal aflibercept injection for neovascular age-related macular degeneration: ninety-six-week results of the VIEW studies. *Ophthalmology* 2014;121:193–201.
- Korobelnik JF, Do DV, Schmidt-Erfurth U, *et al*. Intravitreal aflibercept for diabetic macular edema. *Ophthalmology* 2014;121:2247–54.
- Jeng KW, Wilgucki J, Halperin S, *et al*. Retina specialists treating age-related macular degeneration recommend different approaches for patients than they would choose for themselves. *Retina* 2014;34:1796–801.
- Silver J. Drugs for macular degeneration, price discrimination, and Medicare's responsibility not to overpay. *JAMA* 2014;312:23–4.
- Trichonas G, Kaiser PK. Aflibercept for the treatment of age-related macular degeneration. *Ophthalmol Ther* 2013;2:89–98.
- Ng DS, Kwok AK, Chan CW, *et al*. Intravitreal bevacizumab: safety of multiple doses from a single vial for consecutive patients. *Hong Kong Med J* 2012;18:488–95.
- Chen YH, Wu PC, Shiea J, *et al*. Evaluation of the sterility, stability, and efficacy of bevacizumab stored in multiple-dose vials for 6 months. *J Ocul Pharmacol Ther* 2009;25:65–9.
- Ornek K, Karahan ZC, Ergin A, *et al*. Bevacizumab sterility in multiple doses from a single-use vial. *Ann Pharmacother* 2008;42:1425–8.
- Marmor MF, Martin LJ, Tharpe S. Osmotically induced retinal detachment in the rabbit and primate. Electron microscopy of the pigment epithelium. *Invest Ophthalmol Vis Sci* 1980;19:1016–29.
- Sturmer WQ, Dowdey AB, Putnam RS, *et al*. Osmolality and other chemical determinations in postmortem human vitreous humor. *J Forensic Sci* 1972;17:387–93.
- Gagajewski A, Murakami MM, Kloss J, *et al*. Measurement of chemical analytes in vitreous humor: Stability and precision studies. *J Forensic Sci* 2004;49:371–4.
- Malik D, Tarek M, Caceres del Carpio J, *et al*. Safety profiles of anti-VEGF drugs: bevacizumab, ranibizumab, aflibercept and ziv-aflibercept on human retinal pigment epithelium cells in culture. *Br J Ophthalmol* 2014;98(Suppl 1):11–16.
- Klettner A, Tahmaz N, Dithmer M, *et al*. Effects of aflibercept on primary RPE cells: toxicity, wound healing, uptake and phagocytosis. *Br J Ophthalmol* 2014;98:1448–52.
- de Oliveira Dias JR, Badaró E, Novais EA, *et al*. Preclinical investigations of intravitreal ziv-aflibercept. *Ophthalmic Surg Lasers Imaging Retina* 2014;45:577–84.
- Rosenfeld PJ. Intravitreal avastin: the low cost alternative to lucentis? *Am J Ophthalmol* 2006;142:141–3.
- Stein JD, Newman-Casey PA, Mrinalini T, *et al*. Cost-effectiveness of bevacizumab and ranibizumab for newly diagnosed neovascular macular degeneration. *Ophthalmology* 2014;121:936–45.
- Raftery J, Clegg A, Jones J, *et al*. Ranibizumab (Lucentis) versus bevacizumab (Avastin): modelling cost effectiveness. *Br J Ophthalmol* 2007;91:1244–6.
- Yannuzzi NA, Klufas MA, Quach L, *et al*. Evaluation of compounded bevacizumab prepared for intravitreal injection. *JAMA Ophthalmol* 2015;133:32–9.

- 20 Paul M, Vieillard V, Roumi E, *et al.* Long-term stability of bevacizumab repackaged in 1 mL polypropylene syringes for intravitreal administration. *Ann Pharm Fr* 2012;70:139–54.
- 21 Signorello L, Pucciarelli S, Bonacucina G, *et al.* Quantification, microbial contamination, physico-chemical stability of repackaged bevacizumab stored under different conditions. *Curr Pharm Biotechnol* 2014;15:113–19.
- 22 Palmer JM, Amoaku WM, Kamali F. Quality of bevacizumab compounded for intravitreal administration. *Eye* 2013;27:1090–7.
- 23 Thomas M, Mousa SS, Mousa SA. Comparative effectiveness of aflibercept for the treatment of patients with neovascular age-related macular degeneration. *Clin Ophthalmol* 2013;7:495–501.
- 24 Ahn SJ, Ahn J, Park S, *et al.* Intraocular pharmacokinetics of ranibizumab in vitrectomized versus nonvitrectomized eyes. *Invest Ophthalmol Vis Sci* 2014;55:567–73.
- 25 Moisseiev E, Waisbourd M, Ben-Artzi E, *et al.* Pharmacokinetics of bevacizumab after topical and intravitreal administration in human eyes. *Graefes Arch Clin Exp Ophthalmol* 2014;52:331–7.
- 26 Hahn P, Kim JE, Stinnett S, *et al.* Aflibercept-related sterile inflammation. *Ophthalmology* 2013;120:1100–1.
- 27 Bailey C, Aloumanis V, Walker B, *et al.* Stability of preoperative cataract surgery gel in polycarbonate syringes. *Int J Pharm Compd* 2009;13:564–8.