Ziv-afibbercept in macular disease

Ahmad M Mansour,1 Sara I Al-Ghadban,2 Muhammad H Yunis,1 Marwan E El-Sabbah2

ABSTRACT

Background/aims Afibbercept 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-afibbercept. Our purpose is to determine if ziv-afibbercept can be used in AMD and DME without ocular toxicity, to test the stability of ziv-afibbercept, 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-afibbercept (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-afibbercept 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-afibbercept had no loss of anti-VEGF activity when kept at 4°C in polysorbate 20 for 4 weeks. Similar to bevacizumab, compounded ziv-afibbercept would yield a tremendous saving compared with afibbercept or ranibizumab.

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

Trial registration number NCT02173873.

INTRODUCTION

Afibbercept (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 afibbercept’s high binding affinity for VEGF may impart greater durability of activity and similar efficacy compared with ranibizumab1 or bevacizumab. Afibbercept 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 afibbercept is given bimonthly after 3 monthly injections for eyes with wet AMD. Because of the high cost of ranibizumab and afibbercept, a majority of ophthalmologists worldwide tend to treat patients with bevacizumab4 5 at a major saving for the patient. Commercially, a much cheaper yet identical fusion protein to afibbercept is ziv-afibbercept. Ziv-afibbercept (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-afibbercept can be used instead of afibbercept in certain retinal disorders. Hence the need to answer some major safety concerns: first the difference in osmolarity, and second whether ziv-afibbercept could impair retinal function and alter morphology.6 A preliminary study was conducted on the use of ziv-afibbercept in patients with exudative AMD or diabetic macular oedema (DME) with poor vision. In addition, we tested the stability of ziv-afibbercept over a period of 4 weeks and the economic implications of the use of compounded drug.

METHODS

Ziv-afibbercept is supplied in single-use vials of 100 mg per 4 mL and 200 mg per 8 mL formulated as 25 mg/mL ziv-afibbercept 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 afibbercept (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-afibbercept 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 podivone 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 V6.7, 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
through the fovea 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 pericocular 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 V.20.0 (IBM/SPSS Inc, Chicago, Illinois, USA). Paired sample t test was used to analise the difference between the 1-week outcome and baseline values for visual acuity and CMT.

In vitro ELISA testing of VEGF binding
Ziv-afibercept 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-afibercept 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-afibercept samples (10–10 mg/mL) stored were co-incubated with rhVEGF (100 ng/mL) diluted in phosphate-buffered saline. The ziv-afibercept-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. Following a wash to remove unbound complex, An antibody for VEGF (biotin-conjugated) was then added. 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-afibercept 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. Intravitreal 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-afibercept was 25 mg/mL (and in the current study) and this coincided with the known therapeutic dose (10–40 mg/mL) for afibercept. For that reason, we chose the highest concentration at 10 mg/mL. The stability of ziv-afibercept was assessed by its efficiency to capture rhVEGF by measuring the concentration of free VEGF not bound to complex. At 10 mg/mL, ziv-afibercept 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-afibercept. At 10 μg/mL, ziv-afibercept 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-afibercept to capture free VEGF in clinical settings. Ziv-afibercept 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-afibercept than for afibercept, if the 4 mL ziv-afibercept vial is divided into 40 aliquots (table 3).

| Table 1 Clinical profile of the ziv-afibercept study |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Patient number** | **Race** | **Age** | **Initial vision** | **Final vision** | **Initial CMT in microns** | **CMT 1 week after injection** | **Height of RPE detachment (initial to final, μ)** | **Subretinal fluid** | **Prior therapies** | **Systemic disease** |
| Case 1 (AMD) | Caucasian Male | 80 | 20/800 | 20/150 | 331 | 255 | 833–500 | Yes | No | No |
| Case 2 (AMD) | Caucasian Female | 89 | 20/100 | 20/60 | 748 | 473 | 1040–833 | Yes (minimal) | No | HTN; CAD |
| Case 3 (AMD) | Caucasian Male | 84 | 20/800 | 20/200 | 213 | 181 | 271–90 | Yes (minimal) | No | DM; CAD; smoker |
| Case 4 (AMD) | Caucasian Male | 86 | 20/400 | 20/200 | 372 | 347 | 189–170 | Yes (minimal) | No | HTN |
| Case 5 (diabetic maculopathy) | Caucasian Male | 69 | 20/800 | 20/100 | 508 | 443 | None | Yes | No | DM |
| Case 6 (diabetic maculopathy) | Caucasian Male | 61 | 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.
This can be achieved in two ways: direct puncture of the vial for each patient as recommended by Ng et al. 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-afibercept; second, a less intensive dosing regimen with ziv-afibercept (8 doses for ziv-afibercept or alibfercept 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-afibercept; however, it undergoes a different purification process and contains different buffer solutions that are less irritating when injected intravitreally. Since ziv-afibercept and aflibercept have an identical molecular structure, we decided to test ziv-afibercept (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-afibercept has an osmolarity of 1000 mOsm/kg. Marmor et al. showed in rabbits and primates that solutions of less than 500 mOsm caused no retinal pigment epithelium damage. Toxicity 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-afibercept human injection group relates to the small volume of the drug injected that did not result in a major shift in the total osmality 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 osmality of 312 mOsm/kg or a 4% increase; ie, within the physiological range) and does not affect the retina. Ziv-afibercept 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-afibercept in animal eyes and cell preparation. In a study by Malik et al., 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-afibercept showed mild mitochondrial toxicity at clinically relevant doses. In another study by Klettner et al., aflibercept displayed no cytotoxicity on retinal pigment epithelium cells while it impaired the phagocytic capacity of these cells. Recently data emerged showing that ziv-afibercept was safe in rabbit eyes: 18 rabbits were given intravitreal injection of 0.05 mL ziv-afibercept 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>
<thead>
<tr>
<th>Drug (company)</th>
<th>Ranibizumab (Genentech; Novartis)</th>
<th>Bevacizumab (Genentech; Roche)</th>
<th>Aflibercept (Regeneron; Bayer)</th>
<th>Ziv-afibercept (Bayer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>48 kDa</td>
<td>149 kDa</td>
<td>115 kDa</td>
<td>115 kDa</td>
</tr>
<tr>
<td>Half-life (serum)</td>
<td>4.75 days</td>
<td>8.25 days</td>
<td>18 days</td>
<td>18 days</td>
</tr>
<tr>
<td>Affinity to VEGF165</td>
<td>Kd 46 Pm*</td>
<td>Kd 58 Pm</td>
<td>Kd 0.49 Pm</td>
<td>Kd 0.49 Pm</td>
</tr>
<tr>
<td>Inhibit</td>
<td>VEGF-A</td>
<td>VEGF-A</td>
<td>VEGF-A; VEGF-B; PIGF</td>
<td>VEGF-A; VEGF-B; PIGF</td>
</tr>
<tr>
<td>Vial cost</td>
<td>£741†</td>
<td>£42.74†</td>
<td>£816§</td>
<td>£295.7†</td>
</tr>
<tr>
<td>Cost per eye</td>
<td>£741†</td>
<td>£40†</td>
<td>£816</td>
<td>£48</td>
</tr>
<tr>
<td>Cost per year</td>
<td>£892</td>
<td>£480</td>
<td>£5528</td>
<td>£384</td>
</tr>
<tr>
<td>Vial dose and volume</td>
<td>2.3 mg in 0.23 mL</td>
<td>100 mg in 4 mL</td>
<td>4 mg in 0.1 mL</td>
<td>100 mg in 4 mL</td>
</tr>
<tr>
<td>AMD regimen</td>
<td>0.5 mg monthly</td>
<td>1.25 mg monthly</td>
<td>2 mg monthly 3 times then bimonthly</td>
<td>1.25 mg monthly 3 times then bimonthly</td>
</tr>
<tr>
<td>Injection volume</td>
<td>0.05 mL</td>
<td>0.05 mL</td>
<td>0.05 mL</td>
<td>0.05 mL</td>
</tr>
<tr>
<td>No. injections in year 1</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

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

*Affinity expressed as equilibrium dissociation constant (Kd) in the picomolar (Pm) range.
§NICE technology appraisal guidance 305 issued February 2014 (http://guidance.nice.org.uk/ta305);
Aflibercept is a humanized full-length anti-VEGF antibody produced 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-afibercept showed mild mitochondrial toxicity at clinically relevant doses. In another study by Klettner et al., aflibercept displayed no cytotoxicity on retinal pigment epithelium cells while it impaired the phagocytic capacity of these cells. Recently data emerged showing that ziv-afibercept was safe in rabbit eyes: 18 rabbits were given intravitreal injection of 0.05 mL ziv-afibercept 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.
In VIEW 1 and 2, patients were randomised to one of four groups: 2 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-afibercept (halfway between the 2 mg and 0.5 mg doses studied for aflibercept). Hence the current dose for ziv-afibercept falls within the proven therapeutic doses of aflibercept. An alternative solution is to inject 0.08 mL of ziv-afibercept to deliver a total of 2 mg of the drug.

Currently, bevacizumab is the most cost-effective medication for control of wet AMD compared with ranibizumab (40 times cheaper) or aflibercept. Yannuzzi et al performed a prospective in vivo study of syringes containing intravitreal preparations of bevacizumab from several compounding pharmacies in the USA. Similar findings were previously reported in pharmacies in France, Italy and the UK. There were no microbial contaminants or endotoxin detected in any of the samples. Either sharing multiple doses of ziv-afibercept from a single vial or using compounded ziv-afibercept can substantially reduce the cost of treatment and with a softer regimen of 8 injections instead of 12 injections in the first year, ziv-afibercept would appear theoretically to be more cost effective than bevacizumab (table 3). Like aflibercept, ziv-afibercept is formulated in hypertonic sucrose, a condition that theoretically prevents its intraocular use because hypertonic preparations could damage the retina. According to Silver, the manufacturing differences between intraocular aflibercept and intravenous ziv-afibercept 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-afibercept with multiple injections unlike with single injections. Additional concerns relate to previous reports of aflibercept-related intraocular inflammation and it is likely that the use of ziv-afibercept 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-afibercept 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-afibercept 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. Future clinical studies can reaffirm the long-term safety and efficacy of ziv-afibercept 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-afibercept. Ziv-afibercept 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.

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**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, 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.

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