A Phase 1 Study of KH902, a Vascular Endothelial Growth Factor Receptor Decoy, for Exudative Age-Related Macular Degeneration

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Purpose: To determine the safety, tolerability, and bioactivity of KH902, a fully human fusion protein containing key domains from vascular endothelial growth factor receptors 1 and 2 with human immunoglobulin Fc.

Design: Prospective, single-center, open-label, dose-escalating, interventional case series.

Participants: Twenty-eight patients with choroidal neovascularization (CNV) resulting from exudative age-related macular degeneration (AMD) with lesion size of 12 disc areas or less and best-corrected visual acuity (VA) of 55 letters or worse.

Methods: A single intravitreal injection of KH902 at 1 of 6 escalating doses if no dose-limiting toxicity (DLT) occurred through postinjection day 14 of the previous dose level. Follow-up examinations were performed on postinjection days 1, 3, 5, 7, 14, 28, and 42. The primary end point was at 42 days, and patients were monitored for an additional 6 weeks (12 weeks total).

Main Outcome Measures: The primary safety measures were changes from baseline in VA, intraocular pressure (IOP), intraocular inflammation, and production of anti-KH902 antibody. Dose-limiting toxicity was defined as intraocular inflammation, elevated IOP, significantly reduced vision, or retinal hemorrhage within 42 days after injection. Bioactivity measures included mean change from baseline in VA, central retinal thickness, and total macular volume on optical coherence tomography and CNV changes on fluorescein angiography.

Results: All patients completed the study with no DLT and no serious or drug-related adverse events. Ocular adverse events were mild to moderate in severity, including transient IOP elevation and injection-site subconjunctival hemorrhage after KH902 injections. No serum anti-KH902 antibodies were detected. On day 42 after injection, the mean change in VA from baseline was +19.6 letters with no subjects losing 1 letter or more and 57% of patients gaining 15 letters or more from baseline. The mean change in center point thickness from baseline was –77.2 µm and the mean decrease in CNV area was 12.6%.

Conclusions: No safety concerns were detected after a single, intravitreal injection of KH902 up to 3.0 mg in this phase 1 study. Bioactivity of KH902 was suggested with improvements in VA, reduction in central retinal thickness, and a decrease in CNV area in patients with CNV resulting from exudative AMD, indicating that further study is warranted.

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Age-related macular degeneration (AMD) is the leading cause of irreversible severe vision loss among the elderly in the developed world and is 1 of 3 major cause of blindness in developing countries.1,2 Most of this vision loss results from the neovascular or exudative form of AMD. Ocular neovascularization, including the choroidal neovascularization (CNV) seen in exudative AMD, and increased vascular permeability have been associated with vascular endothelial growth factor (VEGF), a diffusible, secreted protein that is central to the sequence of events leading to vascular leakage and angiogenesis.3–5 The VEGF gene family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PIGF)-1 and PIGF-2. It has been demonstrated that VEGF is an important therapeutic target for treatment of CNV resulting from AMD.6–9

Several VEGF antagonists have been developed and tested in patients with neovascular AMD. Pegaptanib (Macugen; Eyetech Pharmaceuticals, Inc., New York, NY) is a pegylated RNA aptamer that specifically binds the VEGF165 isoform, but no other isoforms of VEGF-A. Intraocular injection of pegaptanib every 6 weeks for 1 year reduced the percentage of patients with classic CNV resulting from AMD.
AMD who experienced moderate vision loss (loss of ≥15 Early Treatment Diabetic Retinopathy Study [ETDRS] letters) from 45% in the sham injection group to 30% in the pegaptanib group. Only 6% percent of patients treated with pegaptanib, compared with 2% in the sham injection group, had a moderate improvement in vision (gain of ≥15 ETDRS letters).

Ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA) is an affinity-matured, humanized Fab fragment of a mouse monoclonal antibody to VEGF that binds all isoforms of VEGF-A. Monthly intraocular injections of ranibizumab in AMD patients with occult or minimally classic subfoveal CNV reduced the percentage of patients with moderate vision loss over 12 months from 38% in the sham injection group to 5% in the ranibizumab-treated group in the pivotal phase 3 trial, and the percentage of patients who experienced moderate visual gain increased from 4.6% to 34%. Taken together, these trials suggest that antagonism of all isoforms of VEGF-A in AMD patients with CNV can result in stabilization of, and often improvement in, vision and confirms that VEGF-A is a very important target in the treatment of neovascular AMD.

Placental growth factor is another member of the VEGF family that contributes to ocular neovascularization and excessive vascular permeability, providing a rationale for targeting multiple VEGF family members and not just VEGF-A. A soluble VEGF receptor decoy, VEGF Trap-Eye (Regeneron Pharmaceuticals, Inc., New York, NY), in which binding domains of VEGF receptors 1 and 2 are combined with the Fc portion of immunoglobulin G, has been shown to be well tolerated and biologically active in exudative AMD and diabetic macular edema. Vascular endothelial growth factor Trap-Eye has a high affinity for all VEGF-A isoforms, PlGF 1 and 2, VEGF-B, VEGF-C, and VEGF-D. Thus, VEGF Trap-Eye is distinguished from ranibizumab by its higher potency for neutralization of all VEGF-A isoforms and its ability to inhibit other related proangiogenic and propermeability VEGF family members.

Similar to VEGF Trap-Eye, KH902 is a recombinant, soluble, VEGF receptor protein in which the binding domains of VEGF receptors 1 and 2 are combined with the Fc portion of immunoglobulin G. The receptor portion of the molecule has a very high affinity for all VEGF-A isoforms, PlGF 1 and 2, and VEGF-B (data not published). The difference between KH902 and VEGF Trap-Eye is that the former also contains domain 4 of VEGF receptor 2. Previous studies have demonstrated that the domain 4 of VEGF receptor 2 is essential for receptor dimerization and enhances the association rate of VEGF to the receptor. Studies have shown that poor pharmacokinetic properties for a fusion protein may be related to a high positive charge of the protein. Because the fourth domain of VEGF 2 has a lower isoelectric point, the addition of this domain to KH902 decreases the positive charge of the molecule and results in decreased adhesion to the extracellular matrix. Preclinical studies have demonstrated that KH902 binds and neutralizes VEGF-A and all its isoforms with a higher binding affinity than ranibizumab. It also binds PlGF, to which ranibizumab has no binding activity. Intravitreal administration of KH902 has been shown to inhibit the formation and development of CNV in a monkey model. Based on these studies, a phase 1 safety study was initiated. This article reports the results of this prospective, open-label, dose-escalation, phase 1 safety study evaluating the safety, pharmacokinetics, biological activity, and maximum tolerated dose of intravitreally administered KH902 in patients with CNV resulting from AMD.

**Patients and Methods**

**Study Design**

A prospective, open-label, single-center, single-dose, dose-escalation, phase 1 study was performed at the West China Hospital, Chengdu, Sichuan, China, from April 2008 to May 2009 in subjects with CNV resulting from neovascular AMD who met the following key inclusion criteria (Table 1, available at http://aaojournal.org): primary or recurrent subfoveal CNV resulting from AMD and best-corrected visual acuity (BCVA; ETDRS protocol) in the study eye of 55 letters or worse (<20/100 Snellen equivalent).

Six dose levels of KH902 (0.05, 0.15, 0.5, 1.0, 2.0, and 3.0 mg) were investigated in a dose-escalation paradigm in which 3 subjects were enrolled in each dose cohort except cohorts 3, 4, and 5 (dose groups of 0.5, 1.0, and 2.0 mg per eye), in which the pharmacokinetic parameters for serum levels of KH902 and plasma levels of VEGF were evaluated. There was a 2-week waiting period after the previous dose cohort was enrolled before enrollment of the next dose cohort was begun to evaluate for any evidence of dose-limiting toxicity (Table 2, available at http://aaojournal.org). The maximum tolerated dose was met if more than 2 patients within a dose group of 6 patients experienced dose-limiting toxicity within 14 days after KH902 injection.

Eligible patients received an intravitreal injection of KH902 (Chengdu Kanghong Biotech, Inc., Chengdu, Sichuan, China) supplied in a liquid vial. All intravitreal injections were performed by a retina specialist following the exact procedure described in the product insert of ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA). Briefly, levofloxacin was instilled 4 times daily for 3 days before injection. The study eye then was anesthetized with either a subconjunctival injection or topical lidocaine. A wire lid speculum was used to insure a sterile field. The various doses of KH902 in a volume of 50 μl (0.05 ml) were delivered intravitreally through a 30-gauge needle, 3 to 4 mm back from the limbus depending on the phakic status of the patient. Perfusion status of the eye was evaluated with indirect ophthalmoscopy and by measuring intraocular pressure (IOP) 5 and 30 minutes after injection. After the injection, the patient was instructed to continue to use the antibiotic drops for the next 4 days.

**Study Assessments and Activities**

Follow-up examinations were performed on postinjection days 1, 3, 5, 7, 14, 28, and 42. Subjects were evaluated at all follow-up visits for safety and tolerability using the following assessments: BCVA measurement, slit-lamp examination, applanation tonometry, indirect ophthalmoscopy, vital signs, physical examination, review of concomitant medications, adverse event reporting, se-
rum electrolytes, creatinine, quantitative protein determination in 24-hour urine specimens, and measurement of serum neutralizing antibodies directed against KH902. Intraocular inflammation was assessed using a standardized grading scale (Table 2, available at http://aaojournal.org). A dose-limiting toxicity was defined as any Common Terminology Criteria for Adverse Events grade 3 or 4 toxicity or grade 2 or 3 ocular toxicity (Table 2, available at http://aaojournal.org). Any grade 1 or 2 toxicity that resulted in dose reduction or discontinuation of the study drug was considered a potential dose-limiting toxicity to be reviewed by the Study Director. Serum anti-KH902 antibody levels also were measured on postinjection days 14, 28, and 42 by surface plasmon resonance technology using a BIACORE 3000 (Amersham Bioscience, Uppsala, Sweden) as described previously.18

Biological activity was assessed by evaluating the mean change from baseline in BCVA, central retinal thickness on optical coherence tomography (OCT), and CNV characteristics on fluorescein angiography. Visual acuity was measured using the ETDRS charts (Precision Vision, La Salle, IL) at 2 m by trained study personnel masked to the study eye. Optical coherence tomography analysis was performed using a Stratus OCT (Carl Zeiss Meditec, Dublin, CA) by trained personnel masked to the study eye on postinjection days 7, 14, 28, and 42. The OCT scan protocol consisted of a 6-radial line scan with the crosshair pattern centered through the fovea. Automated analysis of the 9 ETDRS subfields using the Stratus analysis package to determine center point thickness and total macular volume was performed at the central Reading Center at the West China Hospital. Fluorescein angiography was performed by trained photographers using an FF4 fundus camera (Carl Zeiss Meditec, Oberkochen, Germany) attached to an Ophthavision Imaging System (MRP Group, Boston, MA) capture station. A modified fluorescein angiography acquisition protocol was used for image acquisition, and compliance was monitored by a site visit. The digital images were sent to the Reading Center at the West China Hospital and were analyzed using EyeRoute Proview software (version 6.1; Anka System, Inc., McLean, VA) by 3 independent masked investigators. The fluorescein angiograms were graded for evidence of disease activity, including blood and pigment epithelial detachment, change in lesion size, CNV leakage, and area of subretinal fluid on postinjection days 1, 3, 5, 7, 14, 28, and 42 (from no hyperfluorescence as grade 1 to hyperfluorescence without leakage, to early hyperfluorescence or mid transit and late leakage, to transit bright hyperfluorescence with leakage beyond the borders of the treated area as grade 4).19 Each fluorescein angiogram was analyzed further using advanced image segmentation techniques for edge detection to determine the lesion size and maximum area and extent of leakage. Lesion refers to the area encompassing all lesion components such as CNV (classic, occult, or both), blood, hypofluorescence not from visible blood, serous detachment of the RPE, and hyperfluorescent staining of fibrous tissue.

Ethical Considerations

The study was conducted in compliance with the declaration of Helsinki, China GCP Regulations, and the Harmonized Triparties Guidelines for Good Clinical Practice. The study was reviewed and approved by the local institutional review board of West China Hospital (Chengdu, Sichuan, China). Each study subject had comprehensive discussions with the investigator and gave written informed consent before study entry.

Statistical Methods

All data were collected on case report forms and were analyzed using an intent-to-treat analysis with last observation carried forward on SAS software (SAS Inc., Cary, NC). Data were analyzed using the paired Student t test for changes in mean measurements, with values less than 0.05 considered statistically significant.

Results

Baseline Characteristics

Twenty-eight subjects were included in the study. Enrollment was stopped when the dose reached 3.0 mg per eye and no dose limiting toxicity was observed. The baseline demographic characteristics are shown in Table 3 (available at http://aaojournal.org). Seventy-five percent of subjects were male. The mean age was 69 years. The median baseline BCVA was 20.6 letters (Snellen equivalent, 20/160), and 22 of 28 patients had VA of less than 34 letters (legally blind). Eighty-four percent of subjects were treatment naive. Four of the 28 subjects received previous therapy for AMD in the study eye, including 1 verteporfin (Visudyne; Novartis Pharmaceuticals, Basel, Switzerland) photodynamic therapy treatment, 2 transpupillary thermotherapy treatments, and 1 bevacizumab (Avastin; Genentech, South San Francisco, CA) injection. The mean baseline center point thickness and total macular volume were 336.5 ± 130.5 μm and 7.5 ± 1.7 μm², respectively. The baseline CNV lesions were classified as minimally classic in 80% of patients, predominantly classic in 4% of patients, and occult with

### Table 6. Mean (±Standard Deviation) Best-Corrected Visual Acuity Change from Baseline

<table>
<thead>
<tr>
<th>Dose Group (mg)</th>
<th>Study Day*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 mg (n = 4)</td>
</tr>
<tr>
<td>1</td>
<td>2.00 ± 2.45</td>
</tr>
<tr>
<td>3</td>
<td>10.75 ± 4.19</td>
</tr>
<tr>
<td>5</td>
<td>18.50 ± 1.73</td>
</tr>
<tr>
<td>7</td>
<td>23.50 ± 3.87</td>
</tr>
<tr>
<td>14</td>
<td>26.50 ± 6.60</td>
</tr>
<tr>
<td>28</td>
<td>24.50 ± 6.61</td>
</tr>
<tr>
<td>42</td>
<td>29.25 ± 4.57</td>
</tr>
</tbody>
</table>

BCVA = best corrected visual acuity.

Changes in study eye BCVA measured by best-corrected change in letter score compared with baseline using the Early Treatment Diabetic Retinopathy Study charts.

*Post-treatment day follow-up period.
no classic component in 16% of patients. Neovascular AMD also was present in the fellow eye in 39% of subjects, and none of these eyes were treated with any anti-VEGF agents during the study.

Primary Outcome: Safety and Adverse Events

No patients withdrew from the study. KH902 was well tolerated up to a dose of 3.0 mg. No maximum tolerated dose was reached. Most adverse events were related to the injection procedure and not to the study drug (Tables 4 and 5, available at http://aaojournal.org). All patients experienced an immediate elevation in IOP after the injection, as expected. The average IOP immediately before KH902 injection was 16.25 mmHg, with a mean increase of 10.25 mmHg 5 minutes after the injection (range, 7.67–14.67 mmHg); however, the IOP normalized by 30 minutes after injection in all patients except patient 9, whose IOP returned to baseline by day 3 after injection. One patient in the 0.15-mg group experienced a subconjunctival hemorrhage from the injection procedure.

Intraocular inflammation was evaluated according to the criteria described in Table 2 (available at http://aaojournal.org). No drug-related intraocular inflammation in the study eyes was found after injection of any dose of KH902. There were no cases of endophthalmitis or drug-related systemic serious adverse events. No incidence of cataract formation or progression was detected after intravitreal injection of KH902 in this study. There were no substantial changes in any systemic laboratory test (blood chemistry, hematology, and urinalysis) and no change in vital signs or physical examination findings during the study.

Sera were collected from 28 patients at days 14, 28, and 42 after intravitreal injection of KH902 to detect anti-KH902 antibodies by BIACORE assay. No samples showed positive results for anti-KH902 antibodies, suggesting that KH902, which is composed of full human sequences, has little immunogenic potential in humans.

Visual Acuity

The mean changes in BCVA from baseline in the study eye are summarized in Table 6 and Figure 1. Overall, there was a mean improvement in vision of +19.6 letters by day 42. No subjects exhibited a decline in VA during the course of study, whereas 24 subjects (85.7%) gained visual acuity. At day 42 after injection, 57% of patients improved by 15 letters or more from baseline. There was no significant difference in VA changes between dose groups.

Optical Coherence Tomography

Retinal thickness was measured by OCT at baseline and on days 7, 14, 28, and 42 after KH902 injection (Table 7 and Fig 2). At baseline, the mean center point thickness (CPT) was 336.5±130.5 μm. At day 42 after injection, there was a mean improvement in center point thickness of –77.2 μm. An OCT sample of patient 28 in cohort 6 presented in Figure 3 suggested that central retinal thickness changed from 436 mm on day 0 to 185 mm on day 42 after a single injection of KH902 (Fig 3A). There was no significant difference between treatment cohorts.

Total macular volume is a measure of retinal thickness over a broader area than indicated by center point thickness. Total macular volume was measured by OCT at baseline and on days 7, 14,
Results of a prospective, open-label, dose-escalation phase 1 study investigating the safety and biological effect of KH902 in patients with CNV resulting from AMD when administered by a single intravitreal injection at doses ranging from 0.05 to 3.0 mg.

The primary objective of the study was to assess the safety of a range of KH902 doses and to determine if there is a maximum tolerated dose. This objective was met because KH902 in the study was well tolerated with no local (ocular) or systemic drug-related serious adverse events documented during the study. Moreover, no maximum dose or dose-limiting toxicity was observed during the study. Overall, the most common adverse events were transient elevation of intraocular pressure after intravitreal KH902 injection seen in all patients that normalized within 30 minutes in all patients except one. There were no cases of serious intraocular inflammation or endophthalmitis. One patient experienced a subconjunctival hemorrhage at the injection site. These findings are similar or better than the most common adverse events reported from the published phase 1 study of ranibizumab including subconjunctival hemorrhage at the injection site in 74% of patients (20/27 patients) and sterile intraocular inflammation in 44% of patients (12/27 patients) when the lyophilized ranibizumab, which is not used in the commercial preparation, was used.20 The systemic safety of all dose cohorts was good, with no drug-related serious adverse events observed during the study and a very small amount of the drug reaching the systemic circulation after intravitreal injection (data not shown).

Discussion

Preclinical studies have demonstrated that VEGF is an important stimulus for CNV and that intravitreal delivery of KH902 strongly suppressed CNV.15 This article reports the

Figure 3. Optical coherence tomography and fluorescein angiography images from a patient in cohort 6 with classic choroidal neovascularization with fibrotic lesion (A) at baseline and (B) on day 42 after treatment with an escalating-dose regimen of KH902. Note the central retinal thickness was 436 mm on day 0 (baseline) and improved to 185 mm on day 42 after the intravitreal injection of KH902 at a dose of 3.0 mg per eye. Vision improved from 20/320 on day 0 to 20/200 on day 42, a gain of 9 letters.
A secondary goal of the study was to determine the bioactivity of KH902. The main measures of bioactivity were visual acuity documented by the change in letters on the ETDRS chart, center point thickness assessed by OCT, and lesion or CNV size on fluorescein angiography. On average, the visual acuity improved almost 4 lines at 42 days after a single KH902 treatment, with no notable differences between treatment cohorts in their overall change from baseline. On day 42, no patients lost more than 5 letters from baseline, whereas 57% of patients gained 3 lines or more (≥15 letters). Moreover, the gains in VA seemed durable, with 13 (72%) of 18 patients who continued to be followed up an average of 5 months (range, 3–7 months) after the single KH902 injection maintaining the gain in VA with a mean BCVA improvement of +24.4 letters (data not shown).

All patients treated with KH902 had a reduction in central retinal thickness. A significant reduction in center point thickness was observed by 7 days after KH902 injection. By day 42, the mean reduction in center point thickness and total macular volume was –77.2 μm and –0.6 mm³, respectively. The rapid reduction in retinal thickness and macular volume after KH902 treatment suggests that blockade of VEGF and PIGF results in reduced leakage from CNV. Similar rapid effects were documented in subjects treated with ranibizumab and bevacizumab, and VEGF Trap-Eye.6,22 Reduction in CNV leakage also was seen on fluorescein angiography, with a mean decrease in CNV area of 12%. This was consistent with results seen 1 year after intravitreal injections of pegaptanib and ranibizumab.6,23 After 1 year of pegaptanib treatment every 6 weeks, there was continued growth of CNV, although at a slower rate than that seen in patients receiving sham injection. Although all CNV lesion types seemed to benefit from KH902 therapy, the small number of patients with any given lesion type limits generalization.

In conclusion, no safety concerns were detected after a single intravitreal injection of KH902 up to a maximum single dose of 3.0 mg in this Phase 1 study. No dose-limiting toxicity or maximum tolerated dose were identified. Dose escalation up to 3.0 mg KH902 seemed to result in significant vision improvement. Although the study is limited by a small number of subjects, the results provide further support for the continued investigation of KH902 for the treatment of CNV resulting from exudative AMD.

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References


Footnotes and Financial Disclosures

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Appendix 1. The KH902 Study Group

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