Safety and tolerability of ranibizumab in uni/bilateral neovascular age-related macular degeneration: 12-month TWEEYEs study

Francesco Bandello,1 Giovanni Staurenghi,2 Federico Ricci,3 Edoardo Midena,4 Francesco Viola,5 Tommaso Lupieri Sinibaldi,6 Laura Colombo,6 Elena Peruzzi,6 Stefania Bassanini6

ABSTRACT

Background To evaluate the safety and tolerability of ranibizumab 0.5 mg in patients with uni/bilateral neovascular age-related macular degeneration (nAMD) and best-corrected visual acuity (BCVA)<2/10 and/or second eye affected, regardless of BCVA.

Methods In this 12-month, prospective, multicentre, open-label, single arm, pragmatic intervention study, patients (N=941) aged ≥ 50 years were to receive ranibizumab as per approved label, monthly until maximum stable visual acuity (VA) was achieved (initially, three or more injections may be required). Thereafter, patients were to be monitored monthly for VA and treatment was to be resumed if VA was reduced due to disease activity.

Results Of the 936 patients treated with ranibizumab at least once during the study, 823/113 were unilateral/bilaterally (not simultaneously) treated. The mean (SD) number of ranibizumab injections during the study was 5.4 (2.9)/10.6 (5.0) injections in unilateral/bilaterally treated patients. Three systemic drug-related adverse events (AEs) (all serious, all in unilaterally treated patients) and 18 systemic AE of special interest (AESIs) (11 serious, 16/2 in unilaterally/bilaterally treated patients) occurred during the study. The annual incidence rate (AIR) (events/1000 person-years) for systemic drug-related AEs, considering a 15-day/30-day risk period, was 22.1/19.9. Considering a 30-day risk period, the AIR (events/1000 person-years) for systemic AESIs for unilaterally treated patients was 11.5. Considering a 30-day risk period, the AIR (events/1000 person-years) for ocular drug-related AEs was 23 and AESIs was 11.5.

Conclusions The low incidence of AEs and AESIs demonstrated the good safety and tolerability of ranibizumab in unilaterally/bilaterally treated patients with nAMD in this real-world setting.

INTRODUCTION

AGE-RELATED MACULAR DEGENERATION (AMD)

Age-related macular degeneration (AMD) affects nearly 8.7% of the worldwide population, and the number of patients is expected to increase. In Europe, projections based on recent meta-analysis of AMD prevalence data using the European Eye Epidemiology (E3) consortium show an almost doubling of patients with AMD by 2040, to between 14.9 and 21.5 million with early AMD, and between 3.9 and 4.8 million with late AMD. In Italy, the PAMDI study showed that AMD affects a high percentage of the elderly population; of the 1162 patients aged ≥61 years included in the study, 60.7% had AMD.

AMD is one of the most common causes of permanent central vision loss in the older population aged ≥65 years, predominantly in developed countries. Neovascular AMD (nAMD) accounts for majority of the AMD-associated vision loss, even though it accounts for only 10%-20% of the overall cases of AMD.

Antivascular endothelial growth factor (VEGF) agents are the current standard of care for the treatment of visual impairment due to choroidal neovascularisation (CNV) secondary to nAMD. Ranibizumab 0.5 mg (Lucentis®; Novartis Pharma AG, Basel, Switzerland, and Genentech, South San Francisco, California, USA) was the first anti-VEGF agent to be approved for this indication, in many countries, based on the results from two pivotal trials: Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-Related Macular Degeneration (ANCHOR) and Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration (MARINA) studies. The burden of the second eye developing nAMD is so high that nearly 50% of all eyes at risk would require bilateral treatment by 3 years. However, there are limited data on treatment outcomes in the second-affected eyes, considering most clinical trials include only one study eye per patient, to minimise bias and for statistical reasons.

In Italy, after the authorisation of ranibizumab for nAMD in 2007, limitations were applied on reimbursement. Patients with visual acuity (VA) <2/10 were excluded and for those patients with bilateral disease, the treatment of one eye only was reimbursed; the physician decided which eye to treat, usually the eye with better vision. These patients were therefore excluded from the Lucentis monitoring registry at the Italian Medicines Agency (AIFA) that tracks patients’ eligibility and evaluates the appropriateness of treatment in the approved indications, as well as collecting safety information useful to AIFA to integrate the risk-benefit profile of the drug with data from clinical practice. However, following the approval of ranibizumab 0.5 mg in new indications in 2012, the limitations on the

Correspondence to Dr Francesco Bandello, Department of Ophthalmology, University Vita Salute Hospital San Raffaele, Milano, Italy; bandello.francesco@hsr.it

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reimbursement for patients with VA <2/10 and those with bilateral disease were lifted; hence, the AIFA wanted to assess the safety of ranibizumab 0.5 mg in these specific subgroups.

The TWEYE study (group members of this study are listed in online supplementary appendix 1) was specifically conducted to address this gap, and evaluated the safety and tolerability of ranibizumab 0.5 mg in patients with a VA of <2/10 and those with bilateral nAMD, in a real-life setting in Italy.

MATERIALS AND METHODS

Study design

TWEYE was a 12-month, prospective, multicentre, open-label, single-arm, pragmatic interventional clinical study that was conducted across 107 centres in Italy, from March 2014 to June 2016.

The study protocol was reviewed and approved by the Ethics Committee (EC) of each site and the study was conducted in accordance with the Declaration of Helsinki and applicable local regulatory requirements. Patients provided written informed consent at screening. The study is registered with Clinicaltrials.gov (identifier, NCT01986907).

Patients

Patients aged ≥50 years were included if they fulfilled the following criteria: eyes with best-corrected VA (BCVA) <2/10 due to nAMD (20/100 Snellen equivalent), in both unilaterally and bilaterally affected patients; fellow eye regardless of BCVA in patients with bilateral nAMD, if the first eye had been treated with ranibizumab at least 14 days before (fellow eyes developing nAMD [newly diagnosed] while the study was running were also included in this category).

Key exclusion criteria were active intraocular inflammation (grade trace or above) in either eye; current or suspected ocular or periocular active infection (eg, conjunctivitis, keratitis, scleritis, uveitis, endophthalmitis) in either eye; evidence of retinal pigment epithelial tear at enrolment or vitrectomy in the study eye; ocular disorders that could have confounded the interpretation of results, compromised VA or required medical or surgical intervention during the study period such as mature cataract, retinal vascular occlusion, pre-retinal membrane (macular pucker), retinal detachment or macular hole; uncontrolled glaucoma (intraocular pressure [IOP] ≥30 mm Hg on medication or according to investigator’s judgement) in either eye; systemic treatment with any VEGF inhibitor, including bevacizumab and ziv-aflibercept in the 90 days prior to study enrolment; intraocular treatment with any anti-VEGF or verteporfin photodynamic therapy within 1 month prior to study enrolment; intraocular surgery within 28 days prior to study enrolment; stroke (brain ischaemia), transient ischaemic attack (TIA) or myocardial ischaemia in the 3 months prior to study enrolment; known hypersensitivity to ranibizumab or any other component in the formulation. Pregnant or nursing women and women of childbearing potential not using effective methods of contraception were also excluded.

Study treatment

All patients were to be treated as per the approved ranibizumab label that was in effect at the time of the study (Summary of Product Characteristics [SmPC] 2013)^20^; Treatment was initiated with one injection per month until maximum VA was achieved and/or there were no signs of disease activity that is, no change in VA and in other signs and symptoms of the disease under continued treatment. In patients with nAMD, initially, three or more consecutive, monthly injections may be needed. Thereafter, patients were to be monitored monthly for VA and treatment was to be resumed in case of VA loss due to disease activity. If the patient was already under treatment with ranibizumab at study initiation, treatment during the study was to be given as per approved label (monthly until maximum VA was achieved).

Figure 1  Patient disposition. *Patients who were treated with ranibizumab 0.5 mg at least once and five patients did not received ranibizumab treatment. †Percentages are based on the number of patients enrolled (n=941). VA, visual acuity.
A patient was considered to be unilaterally treated for the whole period if he/she had only one eye treated. When the second eye was also treated, the patient was considered to be bilaterally treated; however, there was an interval of 14 days between treatment of the two eyes.

**Objectives**

The primary objective was to evaluate the annual incidence rates (AIR) of ocular and systemic drug-related adverse events (AEs) and AEs of special interest (AESI). The AESIs were those events possibly related to ranibizumab as per the Periodic Safety Update Report (PSUR) No. 13 and Risk Management Plan (RMP) for ranibizumab (online supplementary appendix 2).

The key secondary objectives included estimating the proportion of systemic and ocular AEs stratifying by type of patient (ie, treated unilaterally or bilaterally) and describing the overall treatment exposure to ranibizumab.

**Assessments**

Safety assessments included collecting information on any AEs throughout the study.

BCVA was to be assessed at baseline and every month, before any other procedure or drug administration, using Snellen VA testing charts.

**Statistical analysis**

It was estimated, based on literature data, that in the worst case the observed incidence rates of eye with ocular-suspected treatment-related AEs in this study should be around 1%, as well as the incidence rate of patients with systemic suspected treatment-related AEs.

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The conservative risk periods related to the ranibizumab injection were defined as 30 days for ocular AEs and both 15 and 30 days for systemic events, based on the vitreous elimination half-life of approximately 2 hours and the systemic to vitreous exposure ratio of 1:90,000.

This in turn would have led to reduction of any bias in the study. Based on these premises, conservative risk periods related to the ranibizumab injections were defined as 30 days for ocular AEs and both 15 and 30 days for systemic events. Events occurring outside of
Of the 941 enrolled patients, 936 (99.5%) patients were included in each analysis based on available assessments and missing data were not replaced. All statistical analyses were produced using SAS for Windows release 9.4 (64-bit) or later (SAS Institute, Cary, North Carolina, USA).

**RESULTS**

**Patient disposition and baseline characteristics**

Of the 944 screened patients, 941 (99.7%) were enrolled and 774 (82.3%) patients completed the study. The main reasons for discontinuation were withdrawal of consent (8.3%) followed by loss to follow-up (4.0%; figure 1). Among the patients that withdrew the consent, there were three patients with serious AE.

Of the 941 enrolled patients, 936 (99.5%) patients were treated with ranibizumab at least once and the remaining five patients did not receive any treatment. All 936 patients were included in the safety set. The ocular safety and systemic safety subpopulations consisted of 908 (97%) and 886 (94.7%) patients, respectively.

At baseline, of the 936 patients included in the safety set, 534 (57.0%) patients had unilateral nAMD, 399 (42.6%) patients had bilateral nAMD and 3 (0.3%) patients were unaffected by nAMD (but had CNV secondary to pathological myopia). During the study, 823 patients (87.9%), all 534 with unilateral nAMD at baseline, 286 with bilateral nAMD and the 3 patients with CNV were treated unilaterally, 57 (6.1%) were treated bilaterally (both eyes enrolled at the same time), and the remaining 56 (6.0%) were first treated unilaterally and subsequently bilaterally (when the other eye developed the disease).

In the total population, the mean age (SD) of patients was 78.7 (7.3) years; 51.6% of the patients were in the age range of 75–84 years. The majority of the enrolled patients were female (62.2%) and Caucasian (99.9%; table 1). A VA ≤2/10 was observed in the treated eye in 89.3% of the 823 unilaterally treated (but not necessarily unilaterally affected) patients; and in both treated eyes in 27.4% of the 113 bilaterally treated patients (online supplementary table 2). Seven hundred and seventy-one of the 1049 treated eyes (73.5%) were naïve to ranibizumab at baseline; of these, 600 were unilaterally treated and 171 were bilaterally treated during the study.

At least one prior or concomitant medical condition was reported in 902 (96.4%) patients; 792 (96.2%) patients in the unilaterally treated group and 110 (97.4%) patients in the bilaterally treated group (online supplementary table 3). The prior medical condition with the highest incidence was cataract operation (n=235, 25.1%), and the active medical condition with the highest incidence was hypertension (n=579, 61.9%; online supplementary table 3).

There were few patients who entered the study without satisfying inclusion criteria while many were included who met the exclusion criteria but did not meet the protocol procedure, and...
There were three systemic drug-related AEs reported during the study, all serious, and all occurred in unilaterally treated patients (table 2). The events of angina pectoris and cerebral haemorrhage, occurred within the 15 day risk period and all three occurred within the 30-day risk period.

The AIR considering a 15-day risk period was 0.0089 (8.9 cases per 1000 person-years; 95% CI 0.0 to 0.0213)) for the overall population and 0.0110 (approximately 11 cases per 1000 person-years; 95% CI 0.0 to 0.0213) overall and 8.5 cases per 1000 person-years (95% CI 0.0 to 0.0181) for the unilaterally treated (figure 2). No systemic drug-related AEs occurred in the bilaterally treated patients, so it was not possible to calculate AIR for this subpopulation. In the self-controlled case series, with a 15-day and a 30-day risk period, the AIR was 7.2 cases per 1000 person-years (95% CI 0.0 to 0.0264) for the unilaterally treated. With a 30-day risk period, the AIR was 7.2 cases per 1000 person-years (95% CI 0.0 to 0.0264) overall and 8.5 cases per 1000 person-years (95% CI 0.0 to 0.0181) for the unilaterally treated (figure 2). The number of events and AIR for the systemic safety subpopulation in sensitivity analyses were comparable with that of the safety population. Systemic AESIs were reported in 18 (1.9%) patients: 16 in unilaterally treated and 2 in bilaterally treated (table 2).

### Annual incidence rates of serious and non-serious systemic drug-related AEs and AES of special interest (Safety set*)

| Incidence rate | Number of events | Patient-time at risk, years | Value (95% CI) | Number of events | Patient-time at risk, years | Value (95% CI) |
|----------------|------------------|----------------------------|----------------|------------------|----------------------------|----------------|
| **Drug-related AEs** | | | | | | |
| **Serious** | | | | | | |
| Overall | 2 | 224.1424 | 0.0089 (0 to 0.0213) | 3 | 415.1732 | 0.0071 (0 to 0.0154) |
| Unilaterally | 2 | 181.0212 | 0.0110 (0 to 0.0264) | 3 | 352.5695 | 0.0085 (0 to 0.0181) |
| Bilaterally | 0 | 43.1211 | 0 (0 to 0) | 0 | 62.6037 | 0 (0 to 0) |
| Non-serious | None | | | | | |
| **AEs of special interest** | | | | | | |
| **Serious** | | | | | | |
| Overall | 3 | 224.1424 | 0.0134 (0 to 0.0285) | 5 | 415.1732 | 0.0120 (0.0015 to 0.0226) |
| Unilaterally | 3 | 181.0212 | 0.0166 (0 to 0.0353) | 5 | 352.5695 | 0.0142 (0.0018 to 0.0266) |
| Bilaterally | 0 | 43.1211 | 0 (0 to 0) | 0 | 62.6037 | 0 (0 to 0) |
| Non-serious | None | | | | | |

*Consisted of all enrolled patients who signed the informed consent, received at least one dose of study drug and had at least one postbaseline safety assessment. Lower limits less than 0 were set to 0.

**AE, adverse event.**
Annual incidence rate of ocular drug-related AEs and ocular AEs of special interest

There were nine ocular drug-related AEs reported during the study (table 4); one (retinal haemorrhage) was serious. Ocular AESI were reported in 11 eyes (1.1%); 6 (0.6%) events were related to ocular hypertension, 4 (0.4%) to intraocular inflammation and 1 (0.1%) to retinal tear (table 4); one event of ocular hypertension was serious but was considered by the investigator to be not related to treatment and the patient was retreated again.

Using a 30-day risk period, there were 10 ocular drug-related AEs (of which one was serious) and five ocular AESIs (of which one was serious). The AIR of ocular drug-related AEs and ocular AESI over a 30-day risk period for the overall population and self-controlled case series are shown in figure 3. The number of AESIs in the risk period than in the control period.

The AIR of serious and non-serious systemic drug-related AEs and AESIs considering a 15-day and 30-day risk period are shown in table 3.

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**Figure 3** Annual incidence rates of ocular drug-related AEs and AESIs—30-day risk period* (Safety set). *Time at risk was defined as the sum of the 30 days following each ranibizumab injection. If a treated eye was given more than one injection, its risk period was defined as: (1) Time elapsed from the injection to the 30 subsequent days—for injections given more than 31 days from the previous; (2) Time elapsed from the first injection to the 30 days following the last injection—for injections given up to 31 days from the previous. †Consisted of all enrolled patients who signed the informed consent, received at least one dose of study drug and had at least one postbaseline safety assessment.

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**Table 4** Ocular drug-related AEs (by Preferred Term) and ocular AEs of special interest during the study (by Safety Risk and Preferred Term; Treated eyes of safety set patients*)

| Preferred term, n (%) | Total (N=1049) |
|-----------------------|---------------|
| Eyes with at least one ocular drug-related AE | 9 (0.9) |
| Conjunctival haemorrhage | 3 (0.3) |
| Foreign body sensation in eyes | 1 (0.1) |
| Ocular discomfort | 1 (0.1) |
| Ocular hypertension | 3 (0.3) |
| Retinal degeneration | 1 (0.1) |
| Retinal haemorrhage† | 1 (0.1) |
| Eyes with at least one ocular AEs of special interest | 11 (1.1) |
| Intraocular inflammation | 4 (0.4) |
| Macular oedema | 1 (0.1) |
| Ocular hyperaemia | 1 (0.1) |
| Retinal oedema | 2 (0.2) |
| Intraocular pressure | 6 (0.6) |
| Ocular hypertension | 6 (0.6) |
| Retinal detachment and retinal tear | 1 (0.1) |
| Retinal tear | 1 (0.1) |

The table summarises ocular AEs that occurred during the study and reported independently from the definition of the risk period. Total number of eyes refers to both eyes of the safety set patients, since AEs were recorded and counted even if occurred on the eye not treated with the study drug. AEs with Site= ’Both eyes’ were counted twice in the present analysis (ie, once for each eye). Each eye could experience more than one AE. Eyes were counted only once in each row. Terms were coded using MedDRA dictionary, version 18.1.

*Consisted of all enrolled patients who signed the informed consent, received at least one dose of study drug and had at least one postbaseline safety assessment.
†Identified as serious AE.
‡Search was performed with and without LLT serous detachment of macula.
AE, adverse event; LLT, Lower-level term; MedDRA, Medical Dictionary for Drug Regulatory Activities.
of events and AIR of drug-related AEs and AESIs for the ocular safety subpopulation in sensitivity analyses are comparable with that of the safety population.

The incidence rate ratio, risk versus control, was 0.8848, indicating a rate of events during the risk period practically equal to that of the control period.

The AIR of serious and non-serious ocular drug-related AEs and AESIs were low (table 5).

### Proportion of ocular and systemic adverse events

Of the 1872 eyes of the safety set patients (both eyes of each patient-treated and not treated), 127 eyes (6.8%) had at least one ocular AE after the first drug injection for a total of 167 ocular events (table 6). The most commonly reported ocular AEs were conjunctivitis (0.8%), cataract (0.7%), conjunctival haemorrhage (0.7%), blepharitis (0.4%) and retinal epithelium detachment (0.3%; table 6). Ocular serious adverse events (SAEs) were reported in three eyes (table 6).

A total of 255 systemic drug-related AEs in 157 patients in the safety set were reported during the study, 209 AEs in 132 unilaterally treated patients and 46 AEs in 25 bilaterally treated patients. Systemic SAEs were reported in 60 (6.4%) patients, unilaterally treated patients and 46 AEs in 25 bilaterally treated patients. Systemic AEs in 1872 eyes of the safety set were reported during the study, 209 AEs in 132 unilaterally treated patients and 46 AEs in 25 bilaterally treated patients. Systemic SAEs were reported in 60 (6.4%) patients, unilaterally treated patients and 46 AEs in 25 bilaterally treated patients.

Ocular AEs in two eyes resulted in treatment discontinuation (retinal pigment epithelium detachment and retinal degeneration) which was considered by the investigator to be treatment-related. Systemic AEs in 15 patients resulted in treatment discontinuation. There were nine deaths reported during the study (pulmonary cancer, sepsis, heart attack, septicaemia, pulmonary embolism, myocardial ischaemia, cardiac failure [all n=1 each], lung cancer [n=2]), but none of them were considered by the investigator to be related to the study drug.

### Discussion

The TWEEYEs study is the first real-life pragmatic study in Italy that assessed the safety and tolerability of ranibizumab 0.5 mg in patients with unilateral or bilateral nAMD and with low VA of <2/10. Ranibizumab treatment was well-tolerated in patients with nAMD. The population included in this trial was of high risk considering that they had a high presence of comorbidities and more than 50% of the patients were 75–84 years of age.

Anti-VEGF agents have revolutionised the treatment of visual impairment in patients with nAMD. Clinical experience in oncology has shown that systemic VEGF inhibition is associated with several ‘classes’ of AEs. Although the safety of intravitreal anti-VEGF agents has been studied extensively, a full understanding of the risk of ocular and/or systemic AEs possibly related to VEGF inhibition after intravitreal administration, especially in high-risk population is still lacking owing to paucity of data. Also, in Italy, the AIFA registry did not routinely capture data for patients with nAMD who had unilateral treatment or had low baseline VA.

Ranibizumab, an anti-VEGF agent specifically designed for intravitreal administration, has a short intrinsic systemic elimination half-life (2 hours), and low serum concentrations (below the concentration necessary to inhibit the biological activity of VEGF by 50%) following intravitreal injection in patients with nAMD. Subsequently ranibizumab does not largely affect plasma VEGF or free VEGF levels after intravitreal administration.

The TWEEYEs study assessed systemic and ocular AESIs (which included all AEs possibly related to ranibizumab as per the PSUR and RMP effective at the time of the study), in addition to AEs with causal relationship established by the investigator, in order to provide the most comprehensive and objective ranibizumab-related AE profile. The AIR of systemic AESIs was 17.8 events per 1000 person-years considering a 15-day risk period following each ranibizumab injection and 16.9 events per 1000 person-years considering a 30-day risk period. Moreover, in TWEEYEs, treating patients in both eyes did not increase the likelihood of systemic events, as systemic AESIs were observed only in two bilaterally treated patients, both events occurred outside of the risk periods. These findings are in line with those reported previously.

Of the known AEs associated with systemic VEGF inhibition, the risk of arterial thromboembolic events are of particular concern. The incidence of stroke/non-myocardial arterial thromboembolic events (0.5% [n=5] including cerebral haemorrhage, cerebrovascular accident and TIA) reported in this study was similar to what was observed in the pooled analysis of four European registries (0.4%).

Consistent with findings from randomised controlled trials (ANCHOR, MARINA and SUSTAIN) and the analysis of four
### Table 7 Systemic adverse events (Safety set*)

| Preferred term, n (%) | Total (n=936) | Unilaterally treated (n=879) | Bilaterally treated (n=113) |
|-----------------------|--------------|-----------------------------|----------------------------|
| Patients with systemic AEs, n (%) | 157 (16.8) | 132 (15.0) | 25 (22.1) |
| Anemia | 4 (0.4) | 3 (0.3) | 1 (0.9) |
| Lymphadenopathy | 1 (0.1) | 0 | 1 (0.9) |
| Arrhythmia | 1 (0.1) | 0 | 1 (0.9) |
| Gout | 1 (0.1) | 0 | 1 (0.9) |
| Abdominal pain upper | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Gastro-oesophageal reflux | 1 (0.1) | 0 | 1 (0.9) |
| Inguinal hernia | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Nausea | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Fatigue | 1 (0.1) | 0 | 1 (0.9) |
| Fever | 10 (1.1) | 8 (0.9) | 2 (1.8) |
| Hepatic lesion | 1 (0.1) | 0 | 1 (0.9) |
| Bronchitis | 9 (1.0) | 7 (0.8) | 2 (1.8) |
| Influenza | 16 (1.7) | 11 (1.3) | 5 (4.4) |
| Pneumonia | 5 (0.5) | 3 (0.3) | 2 (1.8) |
| Femur fracture | 7 (0.8) | 6 (0.7) | 1 (0.9) |
| Fall | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Pancreatic injury | 1 (0.1) | 0 | 1 (0.9) |
| Rib fracture | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Wrist fracture | 1 (0.1) | 0 | 1 (0.9) |
| Hypoglycaemia | 1 (0.1) | 0 | 1 (0.9) |
| Lymphadenopathy | 1 (0.1) | 0 | 1 (0.9) |
| Osteoarthritis | 4 (0.4) | 4 (0.5) | 0 |
| Breast cancer | 1 (0.1) | 0 | 1 (0.9) |
| Prostate cancer treatment | 1 (0.1) | 0 | 1 (0.9) |
| Depression | 3 (0.3) | 2 (0.2) | 1 (0.9) |
| Acute kidney injury | 1 (0.1) | 0 | 1 (0.9) |
| Renal cyst | 1 (0.1) | 0 | 1 (0.9) |
| Chronic obstructive pulmonary disease | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Cough | 4 (0.4) | 4 (0.5) | 0 |
| Pneumothorax | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Respiratory disorder | 1 (0.1) | 0 | 1 (0.9) |
| Respiratory distress | 1 (0.1) | 0 | 1 (0.9) |
| Respiratory failure | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Mastectomy | 1 (0.1) | 0 | 1 (0.9) |
| Blood pressure fluctuation | 1 (0.1) | 0 | 1 (0.9) |
| Peripheral vascular disorder | 1 (0.1) | 0 | 1 (0.9) |
| Varicose vein ruptured | 1 (0.1) | 0 | 1 (0.9) |
| Patients with serious systemic AEs | 60 (6.4) | 51 (5.8) | 9 (8.0) |
| Anaemia | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Goutre | 1 (0.1) | 0 | 1 (0.9) |
| Hepatic lesion | 1 (0.1) | 0 | 1 (0.9) |
| Pneumonia | 5 (0.5) | 3 (0.3) | 2 (1.8) |
| Femur fracture | 7 (0.8) | 6 (0.7) | 1 (0.9) |
| Pancreatic injury | 1 (0.1) | 0 | 1 (0.9) |
| Breast cancer | 1 (0.1) | 0 | 1 (0.9) |
| Prostate cancer recurrent | 1 (0.1) | 0 | 1 (0.9) |
| Chronic obstructive pulmonary disease | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Respiratory disorder | 1 (0.1) | 0 | 1 (0.9) |
| Mastectomy | 1 (0.1) | 0 | 1 (0.9) |
| Patients with non-serious systemic AEs | 111 (11.9) | 90 (10.2) | 21 (18.6) |
| Lymphadenopathy | 1 (0.1) | 0 | 1 (0.9) |
| **Continued**

### Table 7 Systemic adverse events (Safety set*) Continued

| Preferred term, n (%) | Total (n=936) | Unilaterally treated (n=879) | Bilaterally treated (n=113) |
|-----------------------|--------------|-----------------------------|----------------------------|
| Arrhythmia | 1 (0.1) | 0 | 1 (0.9) |
| Bradycardia | 1 (0.1) | 0 | 1 (0.9) |
| Ventricular extrasystoles | 1 (0.1) | 0 | 1 (0.9) |
| Abdominal pain upper | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Intravascular infusion site issue | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Nausea | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Fatigue | 1 (0.1) | 0 | 1 (0.9) |
| Fever | 10 (1.1) | 8 (0.9) | 2 (1.8) |
| Bronchitis | 9 (1.0) | 7 (0.8) | 2 (1.8) |
| Influenza | 15 (1.6) | 10 (1.1) | 5 (4.4) |
| Fall | 1 (0.1) | 0 | 1 (0.9) |
| Rib fracture | 2 (0.2) | 1 (0.1) | 1 (0.9) |
| Wrist fracture | 1 (0.1) | 0 | 1 (0.9) |
| Hypoglycaemia | 1 (0.1) | 0 | 1 (0.9) |
| Arthralgia | 4 (0.4) | 4 (0.5) | 0 |
| Depression | 3 (0.3) | 2 (0.2) | 1 (0.9) |
| Cough | 4 (0.4) | 4 (0.5) | 0 |
| Pneumothorax | 1 (0.1) | 0 | 1 (0.9) |
| Respiratory distress | 1 (0.1) | 0 | 1 (0.9) |
| Respiratory failure | 1 (0.1) | 0 | 1 (0.9) |
| Blood pressure fluctuation | 1 (0.1) | 0 | 1 (0.9) |
| Peripheral vascular disorder | 1 (0.1) | 0 | 1 (0.9) |
| Varicose vein ruptured | 1 (0.1) | 0 | 1 (0.9) |

*Consisted of all enrolled patients who signed the informed consent, received at least one dose of study drug and had at least one postbaseline safety assessment. AEs, adverse event; MedDRA, Medical Dictionary for Drug Regulatory Activities.

European registries,14 15 22 32 there was a low number of ocular events with ranibizumab injections, which might have been caused by the intravitreal injections procedure or by the anti-VEGF effect. The AIR of ocular AEs during a 30-day risk period was 11.5 events per 1000 eye-years and consisted of IOP increase, intraocular inflammation and one case of retinal tear. Overall, the safety findings from this study were comparable to other pivotal clinical trials with no new critical safety findings.14 15 22 32 The mean age of patients enrolled in TWEYES was similar to those enrolled in ANCHOR, MARINA and SUSTAIN studies (mean age of approximately 76–79 years).14 15 32 However, comparison across studies should be interpreted with caution considering the difference in study design, treatment patterns and eligibility criteria.

The mean number of injections per patient of the safety population over 1 year in this trial (6.0) is comparable to the mean numbers reported in the observational LUMINOUS study (5.0 injections)22 and that observed in EPICOHORT (4.4 in the first year), a 2-year, multicentre, open-label, observational, non-comparative study on patients with nAMD from 34 European clinical centres.31 These mean numbers are similar to the mean number of injections (5.6) reported in SUSTAIN, a randomised trial with PRN dosing.32 In a multicentre, nAMD database study conducted in the UK involving 12 951 eyes of 11 135 patients, the mean number of injections in the first year for the first treated eyes was 5.6.16 Observations made in TWEYES study in
real-life setting in Italy showed that in actual clinical practice, the treatment pattern does not necessarily follow the recommended treatment regimen, in spite of treatment being available free of charge. Not all patients received at least three initial injections as recommended in the label, and the proportion of patients receiving injections decreased steadily starting from month 4. Possible reasons for this could be the busy schedules in clinics leading to inadequate patient monitoring and treatment, and the disease not being considered as an emergency condition like, for example, cardiovascular disease. Also, it was observed that many patients who did not meet study entry criteria were included in this study and more than 50% patients were not monitored monthly for VA.

The limitation of the study is that only one fifth of the planned eyes (target 5000 eyes) were enrolled due to a lower than expected enrolment rate. As a result, the CI for the AIR of ocular drug-related AEs was greater (2.85%) than what was planned (0.6%). In addition, there was no systematic gathering of data on prior anti-VEGF medications and this could have led to bias in some patients treated with other medications previously. In addition, previous data related to systemic anti-VEGF therapy used for cancer treatment were not collected systematically leading to a possible bias in this subgroup. The inability to collect systemic AEs in a systematic manner should also be taken into consideration and this is reflective of the considerable differences observed between drug-related AEs, as judged by the investigators, and AIsRs of AESI.

The strength of the study is that it is one of the first studies to enrol a large number of patients for evaluating the safety of ranibizumab 0.5 mg in nAMD, especially in those with VA <2/10 and those with bilateral AMD treatment. The pragmatic study design, method of data collection and statistical analysis were such as to ensure both the study’s internal validity and the applicability of the results to other settings, samples and populations.

In conclusion, the TWEYEi study results confirm the good safety and tolerability profile of ranibizumab 0.5 mg, in both unilaterally and bilaterally treated, mostly elderly patients with nAMD over a 1-year period and in a pragmatic real-world setting characterised by high number of protocol deviations and not all patients were treated as per label. The low incidence of systemic events in this pragmatic clinical study of a predominantly elderly population appear to be consistent with the known low systemic exposure and short half-life of ranibizumab.

Correction notice This paper has been amended since it was published Online First. The TWEYEi study authors have added an acknowledgement to the principal investigators across all the sites who contributed to this study.

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REFERENCES
1 Kawasaki R, Yasuda M, Song SI, et al. The prevalence of age-related macular degeneration in Asians: a systematic review and meta-analysis. Ophthalmology 2010;117:921–7.
2 Klaver CC, Assink JJ, Van Leeuwen R, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam study. Invest Ophthalmol Vis Sci 2001;42:2237–41.
3 Klein R, Klein BE, Cruickshanks KJ. The prevalence of age-related maculopathy by geographic region and ethnicity. Prog Retin Eye Res 1999;18:371–89.
4 Mitchell P, Smith W, Attebo K, et al. Prevalence of age-related maculopathy in Australia. the Blue Mountains Eye study. Ophthalmology 1995;102:1450–60.
5 Wong TY, Wong T, Chakravarthy U, et al. The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. Ophthalmology 2008;115:116–26.
6 Wong WL, Su X, Li X, et al. Global prevalence of age-related maculopathy and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health 2014;2:e106–16.
7 Colin M, Butjinkert GS, Przybora E, et al. Prevalence of age-related macular degeneration in Europe: the past, the future. Ophthalmology 2017;124:1753–63.
8 Piermarocchi S, Segato T, Scopa P, et al. The prevalence of age-related macular degeneration in Italy (PAMD) study: report 1. Ophthalmic Epidemiol 2011;18:129–36.
9 Augood CA, Vingerling JR, de Jong FTVM, et al. Prevalence of age-related maculopathy in older Europeans: the European eye study (EUREYE). Arch Ophthalmol 2006;124:529–35.
10 Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy, the Beaver dam eye study. Ophthalmolog 1992;99:933–43.
Global data on visual impairment in the year 2002. *Bull World Health Organ* 2004;82:844–51.

12 Villegas VM, Aranguren LA, Kovach JJ, et al. Current advances in the treatment of neovascular age-related macular degeneration. *Expert Opin Drug Deliv* 2017;14:273–82.

13 Nowak IZ. Age-related macular degeneration (AMD): pathogenesis and therapy. *Pharmacoil Rep* 2006;58:353–63.

14 Brown DM, Kaiser PK, Michaels M, et al. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. *N Engl J Med* 2006;355:1432–44.

15 Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med* 2006;355:1419–31.

16 Zorzano-Ventura J, Liew G, Johnston RL, et al. The neovascular age-related macular degeneration database: report 2: incidence, management, and visual outcomes of second treated eyes. *Ophthalmology* 2014;121:1966–75.

17 Barthelemes D, Walton RJ, Arnold JJ, et al. Intravitreal therapy in bilateral neovascular age-related macular degeneration. *Ophthalmology* 2014;121:2073–4.

18 Official Journal of the Italian Republic. Available: http://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2007-06-07&atto.codiceRedazionale=07A00906&lenza=30giorni=false [Accessed 29 January, 2018].

19 Official Journal of the Italian Republic. Available: http://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2008-12-18&atto.codiceRedazionale=08A09648 [Accessed 29 January, 2018].

20 Agency EM. Summary of product characteristics. Lucentis 10 mg/ml solution for injection. Novartis 2013. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000715/WC500043546.pdf (Accessed 16 January, 2018).

21 Martin DF, Maguire MG, Ying G-shuang, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med* 2011;364:1897–908.

22 Holz FG, Bandello F, Gillies M, et al. Safety of ranibizumab in routine clinical practice: 1-year retrospective pooled analysis of four European neovascular AMD registries within the luminous programme. *Br J Ophthalmol* 2013;97:1161–7.

23 Xu L, Lu T, Tuomi L, et al. Pharmacokinetics of ranibizumab in patients with neovascular age-related macular degeneration: a population approach. *Invest Ophthalmol Vis Sci* 2013;54:1616–24.

24 Campochiaro PA, Aiello LP, Rosenfeld PJ. Anti-vascular endothelial growth factor agents in the treatment of retinal disease: from bench to bedside. *Ophthalmology* 2016;123:578–588.

25 Kandasamy R, Wickremasinghe S, Guymer R. New treatment modalities for geographic atrophy. *Asia Pac J Ophthalmol* 2017;6.

26 Chen HK, Cick JN. Adverse effects of anticancer agents that target the VEGF pathway. *Nat Rev Clin Oncol* 2009;6:465–77.

27 Zehetner C, Kralinger MT, Modi YS, et al. Systemic levels of vascular endothelial growth factor before and after intravitreal injection of aflibercept or ranibizumab in patients with age-related macular degeneration: a randomised, prospective trial. *Acta Ophthalmol* 2015;93:e154–9.

28 Avery RL. What is the evidence for systemic effects of intravitreal anti-VEGF agents, and should we be concerned? *Br J Ophthalmol* 2014;98(Suppl 1):i7–10.

29 Avery RL, Castellaria AA, Sterline NC, et al. Systemic pharmacokinetics and pharmacodynamics of intravitreal aflibercept, bevacizumab, and ranibizumab. *Retina* 2017;37:1847–58.

30 Pagliarini S, Hatz KB, Margaron F, et al. Similar two-year safety profiles of bilateral versus unilateral treatments with ranibizumab 0.5 Mg in nAMD and DME. poster (1510-B0236) presented at the Association for research in vision and ophthalmology (ARVO). Denver, Colorado, 2015.

31 Pagliarini S, Beatty S, Lipkova B, et al. A 2-Year, Phase IV, Multicentre, Observational Study of Ranibizumab 0.5 mg in Patients with Neovascular Age-Related Macular Degeneration in Routine Clinical Practice: The EPICOHORT Study. *J Ophthalmol* 2014;2014:1–9.

32 Holz FG, Amoako W, Donate J, et al. Safety and efficacy of a flexible dosing regimen of ranibizumab in neovascular age-related macular degeneration: the sustain study. *Ophthalmology* 2011;118:663–71.