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CLINICAL TRIALS

Topical administration of regorafenib eye drops: phase I dose-escalation study in healthy volunteers

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AIM

Regorafenib is a multikinase inhibitor under investigation for use in neovascular age-related macular degeneration. In this phase I study, regorafenib eye drops were administered to healthy volunteers to provide information on safety, tolerability and systemic exposure.

METHODS

This was a single-centre, randomized, double-masked, parallel-group, dose-escalation, placebo-controlled study. Subjects received regorafenib eye drops (30 mg ml^{-1}, 25 μl) as a 0.75 mg single dose (Cohort 1), 0.75 mg twice daily (bid) or thrice daily (tid) over 14 days (Cohorts 2 and 3, respectively), 1.5 mg tid unilaterally for 3 days, then bilaterally for up to 14 days (Cohort 4), or placebo. Plasma samples were taken to estimate systemic exposure. Safety and functional assessments were performed throughout the study.

RESULTS

Thirty-six subjects received regorafenib and 12 received placebo. Regorafenib was safe and well tolerated over the dose range. No pathological changes occurred in the anterior, vitreous or posterior eye compartments. Mild eyelid redness, oedema and conjunctival hyperaemia were observed across all regorafenib cohorts; these were comparable with the effects seen with placebo. Predominant symptoms were blurred vision in the active and placebo groups. Systemic safety evaluations showed no clinically relevant findings. Absolute systemic exposure after multiple administrations of regorafenib eye drops at a dose of 0.75 mg was 600–700-fold lower than after multiple oral administration of 160 mg day^{-1}, the dose approved in cancer indications.

CONCLUSION

These results indicate a favourable safety and tolerability profile of regorafenib eye drops up to 30 mg ml^{-1} tid for use in clinical studies.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Neovascular age-related macular degeneration (nAMD) is currently treated intravitreally by large protein biologics, which target vascular endothelial growth factor (VEGF).
- Regorafenib is a multikinase inhibitor that targets nAMD-relevant kinases, VEGF receptor 2/3 and platelet-derived growth factor.
- Regorafenib was developed as an eye drop formulation for potential use in nAMD.

WHAT THIS STUDY ADDS

- This study provides the first-in-human information on an oily eye drop formulation of regorafenib in healthy subjects.
- The study design addresses both local tolerability and convenience aspects for topical administration to the eye.
- The systemic pharmacokinetics of regorafenib eye drops under conditions of 100% compliance are described.

Introduction

Neovascular age-related macular degeneration (nAMD) is currently treated intravitreally by large protein biologics, which target vascular endothelial growth factor (VEGF), a key mediator in disease progression [1–3]. **Regorafenib** is a multikinase inhibitor that is directed against nAMD-relevant kinases, **VEGF receptor 2/3** [4] and **platelet-derived growth factor receptor β** [5], in addition to other kinases. Regorafenib was initially developed as an oral oncology drug for the treatment of solid organ malignancies and was approved for the treatment of metastatic colorectal cancer (CRC) in 2012 and for locally advanced, unresectable or metastatic gastrointestinal stromal tumours (GIST) in 2013 [6, 7]. Hence, the clinical pharmacology-related properties of regorafenib after oral administration are well characterized.

Regorafenib was recently developed as a topical eye drop formulation for potential use in nAMD. The rationale was that, through inhibition of VEGF receptors 2/3, regorafenib targets the same pathway as that of the currently used intravitreally injected biologics that bind and neutralize the ligand. It was anticipated to provide quality-of-life benefits compared with intravitreal anti-VEGF agents, which cannot be self-administered by the patient. Intraocular injections are invasive and increase the risk of intraocular infections as well as chronic secondary glaucoma. In efficacy studies performed in various species employing the laser-induced choroidal neovascularization model, treatment with regorafenib eye drops was shown to be efficacious (Joussen *et al.*, manuscript in preparation).

Here, we report the findings of a phase I single- and multiple-dose escalation study in healthy volunteers. The main objective of the study was to investigate the local safety profile and to assess the rate and extent of systemic absorption after administration of regorafenib eye drops prior to subsequent clinical studies.

Methods

Drug product and administration

Topical regorafenib suspended in 100% light liquid paraffin (Bayer AG, Pharmaceuticals, Wuppertal, Germany) was used at two different concentrations, at 2% (w/v) (20 mg ml^{-1}) and 3% (w/v) (30 mg ml^{-1}). Placebo comprised 100% liquid paraffin only. One eye drop had a volume of 25 μl, a volume deemed appropriate for quantitative topical application of study drug to the eye surface [8]. Due to the importance of following a highly standardized administration procedure [9], the drug was applied to the subject while in the sitting position with the head tilted backwards, and the application bottle was sustained in a vertical position during drop application. Pharmaceutical tests prior to the start of the clinical study had shown a variability in the amount of drug delivered of approximately 10–15% under identical handling and application conditions. The packaging and labelling were designed to maintain blinding of the investigator and subject. A designated person at the study site who was not involved in study conduct or safety assessments was responsible for treatment application. The protocol and all protocol amendments were reviewed and approved by the independent ethics committee and institutional review board of the study site (Ethik-Kommission der Landesaerztekammer Thueringen, Jena, Germany) before the start of the study and before implementation of the amendments, respectively (SocraTec Study No 1276reg12ct; EudraCT 2013–003709-25).

Dose rationale

The doses were chosen based on preclinical experiments in rodents and nonhuman primates [10, 11] on scaling according to the surface of the eyeballs in the different species, which is required owing to the lipophilicity of regorafenib, and on droplet volume. The expected therapeutic dose was derived from these experiments, and ranged from 2% bid to 3% tid. Using this range, the aim of the present phase I study was to determine the highest tolerable dose to be tested in a phase II study. The cohort in which two drops of 3% tid were administered to both eyes was intended to generate multiples of exposure and explore the potential for future use in both eyes.

Study design

This was a randomized, double-masked, placebo-controlled, sequential ascending-dose phase I safety study. There were four cohorts of 12 healthy male subjects each (nine subjects received regorafenib eye drops and three subjects received placebo), with one cohort per dose level. There were separate randomization lists for each dose level of the study – i.e. one randomization list for the first single dose level, and one randomization list for each of the multiple dose levels. The randomization list included the randomization codes, subject

identifier and treatment assigned. When subjects were assigned for treatment, they received a unique randomization number.

Subjects received placebo or regorafenib eye drops (30 mg ml^{-1}) to the left eye, the latter as either a 0.75 mg single dose (Cohort 1), 0.75 mg twice daily (bid) or thrice daily (tid) over 14 days (Cohorts 2 and 3), or 1.5 mg tid for 3 days to the left eye, then bilaterally up to 14 days (Cohort 4). Subjects could also de-escalate to a lower regorafenib concentration (20 mg ml^{-1}) if the 30 mg ml^{-1} formulation was not tolerated. Dose increases to the next regorafenib level were only implemented if the preceding dose level was deemed tolerated after a data evaluation by an expert medical team that included review of all ocular signs and symptoms, particularly those defining the individual stop criteria (listed in Appendix S1), safety laboratory results and adverse events (AEs). The study data remained blinded until database lock (except for the dose escalation/de-escalation steps, for which the expert medical team was unmasked to perform the safety review). The entire dosing scheme is illustrated in Figure 1. As this was an exploratory study, no formal statistical sample size estimation was performed.

Population

Healthy male volunteers aged 18–45 years were included. Subjects with ocular disease or a history of ocular disease or surgery were excluded, with the exception of myopia, hyperopia or presbyopia. Subjects were required to have no abnormalities in general or on ocular examination. Subjects with a corrected visual acuity of less than 20/20 or intraocular pressure (IOP) >21 mmHg were excluded. Body mass index was required to be within the range of 18–30 kg m^{-2}. Use of contact lenses was not allowed within 1 week of enrolment, or during the study. During the study, participants had to discontinue all medical treatments. Previous or co-medication with drugs affecting the pharmacokinetics of regorafenib (i.e. inhibitors/inducers of cytochrome P450 3A4 or UDP-glucuronosyltransferase 1A9) had to be stopped at least 1 month prior to study start. Smoking was not allowed (former smokers or those who had stopped smoking at least 3 months before the first study drug administration were eligible). Subjects were required to use adequate contraception when sexually active. All subjects provided written informed consent.

Safety and functional ocular assessments

Abnormal findings after baseline were recorded as AEs. Individual listings of AEs were documented. The incidence of treatment-emergent AEs (TEAEs) was summarized by treatment using Medical Dictionary for Regulatory Activities (MedDRA) terms. The following ocular safety assessments were conducted: eyelid redness and oedema, conjunctival hyperaemia and oedema, subconjunctival haemorrhages, corneal abnormalities by ocular surface staining, anterior chamber cells/flare, lens opacity/cataract and vitreous cells/flare.

Standardized scales were used for eyelid redness/oedema and conjunctival hyperaemia [12], corneal abnormalities [13] and anterior chamber cells/flare and vitreous cells/flare [14]. Eyelid redness and oedema and conjunctival hyperaemia were graded on a four-point scale (0 = none/absence, 1 = mild, 2 = moderate and 3 = severe). Conjunctival oedema, subconjunctival haemorrhages, corneal abnormalities and abnormalities of the lens (opacity/cataract) were recorded as either present or absent. Ocular surface staining was graded according to the Oxford Scheme six-point scale [13]. Anterior and vitreous cells and flare were graded according to the Standardization of Uveitis Nomenclature (SUN) grading scale [14].

Other ocular examinations included objective and subjective refraction, best-corrected visual acuity (BCVA) before and at repeated times after treatment, IOP, endothelial cell density count, retinal thickness and structural changes of the retina, and optic nerve before and after treatment. Visual acuity was assessed at 4 m using standardized procedures based on the Early Treatment Diabetic Retinopathy Study

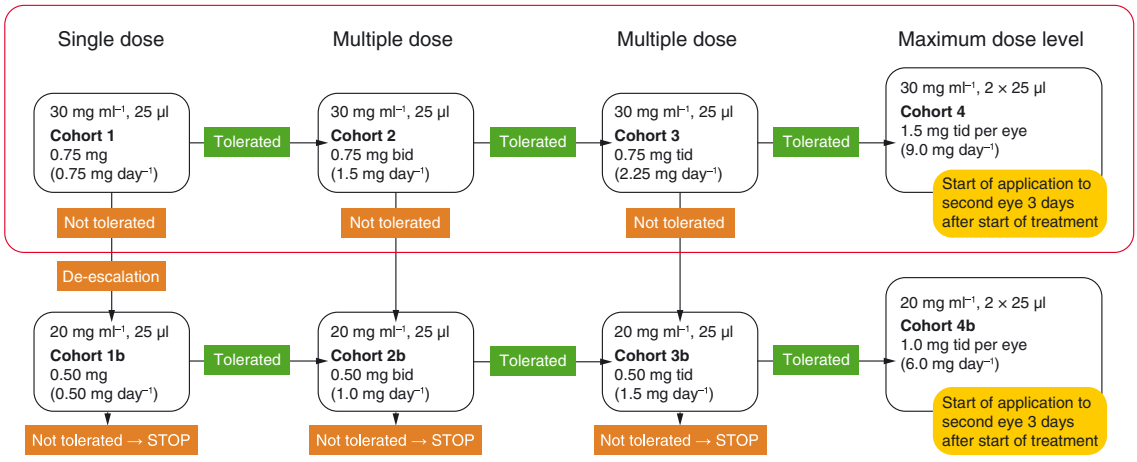

Figure 1

Dosing schedule. Bid, twice daily; tid, thrice daily. The red frame indicates the cohorts that were treated in the study; no dose reduction was necessary

(ETDRS) protocol [15]. The difference from baseline was expressed as the change in ETDRS letters. General safety was assessed through the monitoring of vital signs, laboratory parameters, physical examinations and electrocardiograms. Subjects were confined to the research facility for 15 days, and then returned to the unit repeatedly up to day 28. This was deemed necessary in order to monitor the local and systemic tolerability, and to ensure that systemic exposure to regorafenib was not biased by application mistakes or compliance failure.

Subject rating of treatment

For the assessment of convenience of the treatment, subjects were asked to rate ocular symptoms caused by eye drop instillation (e.g. itching, burning, pain/discomfort, foreign body sensation, photophobia and blurred vision) 5 min after application, on a four-point scale. In addition, the general convenience of eye drop instillation was assessed predose and 20–30 min post-instillation, asking the subjects to rate their answers to the more general question, 'How much pain or discomfort have you had in and around your eyes on a numerical rating scale from 0 to 10'. A score of 0 was defined as 'none' and a score of 10 was defined as 'very severe'. Any rating >0 generated an AE, although this was reported after solicited questioning. For a global assessment at the end of treatment, subjects were asked to respond to the question, 'If you needed to use this medication to treat an eye condition over a period of several months, how happy would you be taking this medication?' on a numerical scale (0 = extremely unhappy to 10 = very happy).

Descriptive statistics were calculated for all variables collected at the screening phase (demographic characteristics, ocular examinations, electrocardiograms, vital signs and laboratory safety tests). During the treatment period, descriptive statistics were calculated for all evaluations of safety and tolerability.

Pharmacokinetics

Systemic exposure to regorafenib was assessed by repeated pharmacokinetic sampling for determination of plasma concentrations of regorafenib and its metabolites M-2 and M-5 [16]. Sampling was performed at several time points from predose until 7 days after a single dose, and 14 days after the last multiple dose.[1] Sampling time points were selected based on preclinical experience and were extensive enough to ensure that the absorption and elimination phases of the compound were adequately described. Plasma concentrations of regorafenib and its metabolites were determined using validated liquid chromatography–tandem mass spectrometry methods [6, 17].

[1]Pharmacokinetic samples were taken predose, and 10, 20,*,** 30*** and 40*,** min; 1, 2, 3, 4, 5, 6, 8, 8.5,*** 9,*** 10, 12, 16, 24 and 32* h; 2, 3, 4,**,*** 5, 7, 10, 13 and 14 days after first or single dosing, as well as 10 and 30 min; 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24 and 32 h; and 2, 3, 5, 7 and 14 days after the last dose (given 14 days after the first dose).
*Only in cohort 1, sampling ended after 7 days; **only after bid dosing; ***only after tid dosing.

Based on the plasma concentration–time data, the following parameters for regorafenib were calculated: area under the concentration–time curve for relevant time intervals according to the respective dosing intervals [e.g. AUC from time zero to time of last measurable concentration ($AUC_{0-tlast}$), AUC measured up to 8 h (AUC_{0-8}), AUC measured up to 12 h (AUC_{0-12})], corresponding average concentration (e.g. C_{av0-12}) and maximum and minimum plasma concentration (C_{max} and C_{min}). In Cohorts 2, 3 and 4, the apparent terminal elimination half-life ($t_{1/2}$), and the accumulation factors (R_A, ratio of exposure after first to last dose) were determined for C_{max} (R_AC_{max}) and for AUC_{0-8} (R_AAUC_{0-8}). For M-2 and M-5, all measured concentrations were below the lower limit of quantification (LLOQ) (2 μg l^{-1}); therefore, no further evaluation was performed.

The non-normalized and dose- and body weight–normalized pharmacokinetic characteristics AUC, AUC_{0-8} and AUC_{0-12}, dose- and body weight-normalized C_{av0-8} and C_{av0-12}, and dose and body weight-normalized C_{max} after first (day 0) and last (day 14) study drug administration were analysed in an explorative manner, assuming log-normally distributed data. To investigate dose proportionality, an exploratory analysis of variance (ANOVA), including the factor dose, was performed on the log-transformed values of the pharmacokinetic parameters. Based on these analyses, point estimates (least-square means) and 90% confidence intervals for the dose ratios in relation to the first dose were calculated by retransformation of the logarithmic results. Pharmacokinetic analyses were performed using WINAE2.80 (Bayer AG, Berlin, Germany) and WinNonlin 5.3 (Pharsight Corporation, Mountain View, CA 94041, USA).

Nomenclature of targets and ligands

Key protein targets and ligands in this manuscript are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [18], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [19].

Results

Participants

Forty-eight subjects received at least one dose of study medication and were included in the safety analysis set. A total of 36 subjects received regorafenib eye drops (n = 9 per cohort) and 12 subjects received placebo (n = 3 per cohort). Baseline characteristics are shown in Table 1. All subjects received active drug as 3% (30 mg ml^{-1}) eye drop formulation, and no de-escalation was necessary.

Safety and tolerability

No local or systemic findings required the dose escalation to be stopped or a change to a lower dose. Ocular TEAEs were reported at similar frequencies for subjects receiving regorafenib or placebo. Most were mild to moderate in severity. No serious AEs were reported. In Cohort 3 (2.25 mg day^{-1}), one subject developed a mild periorbital contact dermatitis on day 2 of dosing. The oedema resolved with continued dosing

Table 1

Baseline and demographic characteristics

	Cohort 1 Regorafenib 0.75 mg day^{-1} (*n* = 9)	Cohort 2 Regorafenib 1.5 mg day^{-1} (*n* = 9)	Cohort 3 Regorafenib 2.25 mg day^{-1} (*n* = 9)	Cohort 4 Regorafenib 9.0 mg day^{-1} (*n* = 9)	Placebo (pool) (*n* = 12)	Total (*n* 1= 48)
Male, *n* (%)	9 (100)	9 (100)	9 (100)	9 (100)	12 (100)	48 (100)
White, *n* (%)	9 (100)	9 (100)	9 (100)	9 (100)	12 (100)	48 (100)
Age, years, mean	29.7	31.0	30.8	34.2	32.6	31.7
BMI (kg m^{-2}), mean	25.3	24.0	24.4	25.5	25.5	25.0
Smoking, *n* (%)						
Former	0	0	1 (11.1)	1 (11.1)	1 (8.3)	3 (6.3)
Never	9 (100)	9 (100)	8 (88.9)	8 (88.9)	11 (91.7)	45 (93.8)

BMI, body mass index

until day 7; however, a very mild flush remained until 2 days after the end of study drug application (day 17). A relationship with the drug could not be excluded, although the skin reaction tended to disappear with continued dosing.

Eyelid redness and oedema, conjunctival hyperaemia and oedema were observed across all regorafenib cohorts; these were mild and comparable with the effects of placebo. No subconjunctival haemorrhages were reported in this study. IOP did not change after treatment, and no increases of IOP above 21 mmHg occurred. Ocular surface staining, which was graded as 0 (no changes) or 1 (mild changes) in most cases, was transient and considered clinically nonsignificant by the investigator. No pathological changes of the anterior eye chamber or lens were reported. No vitreous cells or flares were observed. Retina structures appeared normal prior to and after dosing. Endothelial cell density and retina thickness were unaffected. No clinically relevant changes in laboratory parameters were observed. Regorafenib eye drops did not affect blood pressure or pulse rate. No effect of regorafenib on electrocardiographic parameters was observed.

Functional and subjective assessments

The most frequently occurring ocular symptoms after eye drop administration included blurred vision after solicited questioning (regorafenib 97%; placebo 83%), a transient reduction in visual acuity (regorafenib 31%; placebo 50%), a burning sensation (regorafenib 25%; placebo 17%), itching (regorafenib 17%; placebo 42%), ocular/conjunctival redness (regorafenib 19%; placebo 17%), a foreign body sensation (regorafenib 17%; placebo 8%), increased lacrimation (regorafenib 8%; placebo 17%) and lid oedema (regorafenib 8%; placebo 8%). Pain and discomfort ratings were comparable between regorafenib and placebo, and were mostly 0 (i.e. no pain and discomfort).

Nearly all subjects (94%) had blurred vision at some time point, which mostly started shortly after instillation. Symptoms were transient and lasted for 15–30 min in most subjects. The intensity of blurred vision was mostly mild, but three subjects in Cohort 4 and one in the placebo group reported severe blurred vision. Blurred vision resulted in temporary reductions in visual acuity (≥ 5 ETDRS letters) in 35% of all subjects at some time during the 14-day treatment; reductions were most pronounced 10 min after dosing (Figure 2).

After the last dose of the study drug, the question, 'If you needed to use this medication to treat an eye condition over a period of several months, how happy would you be taking this medication?' was answered positively, with scores mainly between 9 and 10. No difference was apparent between regorafenib (any dose) and placebo (Figure 3).

Pharmacokinetics

The pharmacokinetic data are summarized in Table 2. After the first administration, regorafenib was absorbed slowly, with individual lag times between 4 h and 16 h at LLOQ = 0.1 μg l^{-1}. After administration of regorafenib 0.75 mg single dose (Cohort 1), a mean C_{max} of 0.420 μg l^{-1} was reached after 24 h. Accordingly, maximum concentrations of regorafenib were reached at the end of the respective dosing intervals in the multiple-dose groups, with a geometric mean C_{max} of 0.234 μg l^{-1} and 0.186 μg l^{-1} after regorafenib 0.75 mg bid and tid, respectively, and 0.302 μg l^{-1} after regorafenib 1.5 mg tid. In multiple-dose regimens, plasma concentrations increased in a constant manner until day 7, when steady state was reached, and declined after last dosing with an apparent elimination $t_{1/2}$ of between 60 h and 67 h (see Figure 4).

The geometric mean $AUC_{0–8}$ after first dosing of 0.75 mg regorafenib was 0.302, 0.255 and 0.282 μg·h l^{-1} in cohorts 1–3, respectively, and 0.451 μg·h l^{-1} after regorafenib 1.5 mg, with high variability. $C_{av0–8}$ was 0.0377 μg l^{-1} and 0.0352 μg l^{-1} after regorafenib 0.75 mg (Cohorts 1 and 3, respectively), and 0.0564 μg l^{-1} after regorafenib 1.5 mg (Table 2).

Considerable accumulation was observed between day 0 and day 14 (see Figure 5), with the $AUC_{0–8}$ being 179- and 228-fold higher after multiple than after first dosing in the tid regimens in Cohorts 3 and 4 ($R_AAUC_{0–8}$), respectively. The accumulation ratios for C_{max} (R_AC_{max}) were 24.0, 59.7 and 68.3 (Cohorts 2–4, respectively) (Table 2). These R_A values should, however, be interpreted with caution as they were

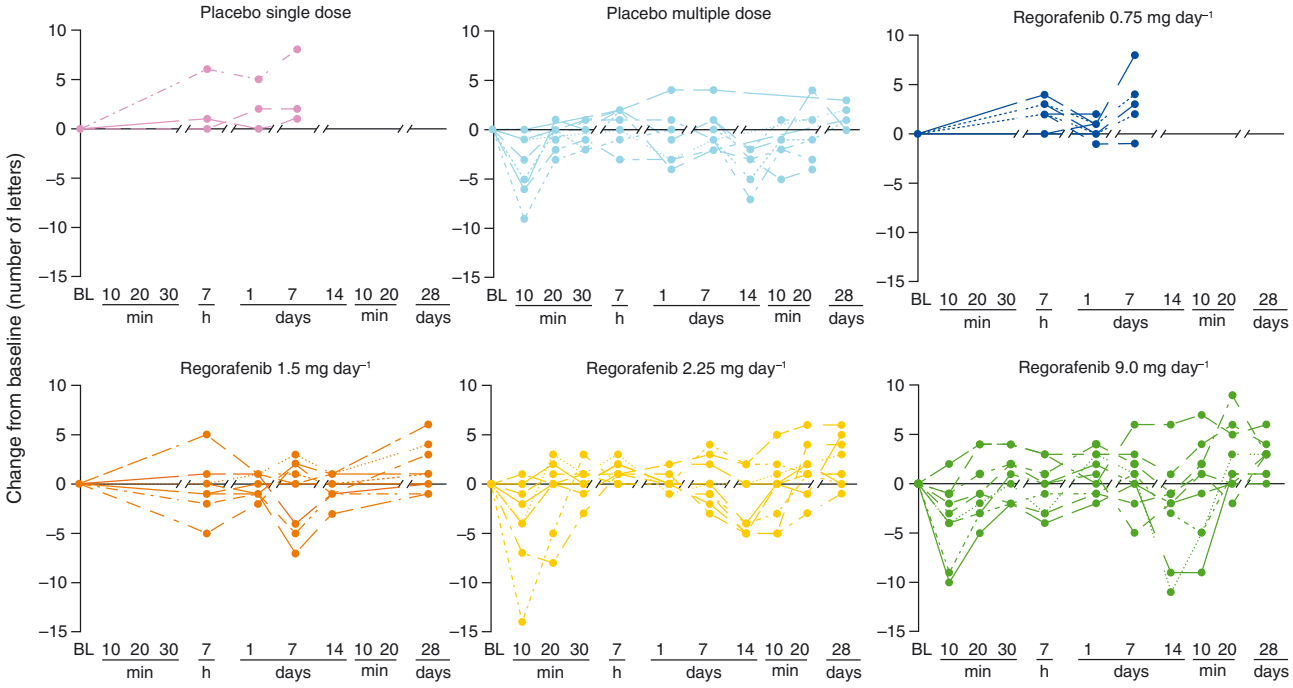

Figure 2

Change from baseline in visual acuity score (Early Treatment Diabetic Retinopathy Study charts) in individual subjects by regorafenib eye drop treatment (0.75 mg day^{-1} single dose; 1.5 mg day^{-1}, 2.25 mg day^{-1} and 9.0 mg day^{-1} multiple dose) or placebo (single or multiple dose). Groups sizes were: n = 12 (placebo) and n = 36 (regorafenib). BL, baseline

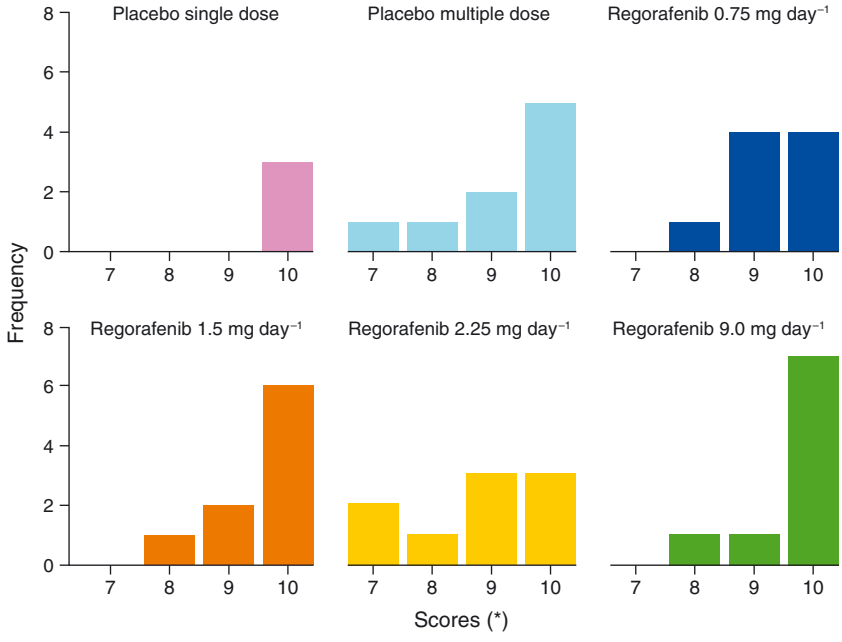

Figure 3

Eye medication acceptance scores 24 h after the end of treatment with regorafenib eye drops (0.75 mg day^{-1} single dose; 1.5 mg day^{-1}, 2.25 mg day^{-1} and 9.0 mg day^{-1} multiple dose) or placebo (single or multiple dose). Groups sizes were n = 12 (placebo) and n = 36 (regorafenib). Subjects were asked to respond to the question, 'If you needed to use this medication to treat an eye condition over a period of several months, how happy would you be taking this medication?' on a numerical scale (*0 = extremely unhappy to 10 = very happy)

Table 2

Pharmacokinetic parameters of regorafenib in the plasma following an eye drop formulation of 0.75 mg single dose, 0.75 mg bid (1.5 mg day^{-1}) and tid (2.25 mg day^{-1}), and 1.5 mg tid (9.0 mg day^{-1}) over 14 days. Data are geometric mean/%CV (range)

A. First dose administration

Parameter	Unit	*n*	Cohort 1 Regorafenib 0.75 mg single dose	*n*	Cohort 2 Regorafenib 0.75 mg bid (1.5 mg day^{-1})	*n*	Cohort 3 Regorafenib 0.75 mg tid (2.25 mg day^{-1})	*n*	Cohort 4 Regorafenib 1.5 mg tid (9.0 mg day^{-1})
AUC_{0-8}	μg·h l^{-1}	6	0.302/96.4 (0.109–0.869)	6	0.255/182 (0.109–1.70)	6	0.282/80.1 (0.117–0.550)	7	0.451/165 (0.101–1.64)
AUC_{0-12}	μg·h l^{-1}	8	0.771/170 (0.118–2.74)	9	0.640/204 (0.109–5.53)	–	n.c.	–	n.c.
C_{max}	μg l^{-1}	9	0.420/94.0 (0.118–1.09)	9	0.234/98.6 (0.104–1.21)	6	0.186/36.1 (0.114–0.312)	7	0.302/95.8 (0.106–0.963)
t_{max}[a]	h	9	24.0 (12.0–120)	9	11.9 (8.00–12.1)	6	7.92 (7.92–7.95)	7	8.00 (6.08–8.02)
C_{av0-8}	μg l^{-1}	6	0.0377/96.4 (0.0136–0.109)	–	n.c.	6	0.0352/80.1 (0.0146–0.0688)	7	0.0564/165 (0.0126–0.205)

B. Last dose administration

Parameter	Unit	*n*	Cohort 2 Regorafenib 0.75 mg bid (1.5 mg day^{-1})	*n*	Cohort 3 Regorafenib 0.75 mg tid (2.25 mg day^{-1})	*n*	Cohort 4 Regorafenib 1.5 mg tid (9.0 mg day^{-1})
AUC_{0-8md}	μg·h l^{-1}	9	27.2/69.3 (12.1–68.2)	9	32.1/102 (8.14–98.2)	9	97.8/48.3 (45.2–232)
$AUC_{0-8md/D}$	h l^{-1}	9	0.0363/69.3 (0.0161–0.0909)	9	0.0428/102 (0.0109–0.131)	9	0.0326/48.3 (0.0151–0.0774)
AUC_{0-12md}	μg·h l^{-1}	9	40.7/65.7 (17.7–101)	–	n.c.	–	n.c.
$C_{max,md}$	μg l^{-1}	9	5.61/69.0 (2.15–12.9)	9	6.86/126 (1.34–27.4)	9	20.5/39.0 (12.5–38.5)
$C_{min,md}$	μg l^{-1}	9	2.45/63.4 (1.07–5.83)	9	2.79/92.0 (0.923–7.83)	9	8.32/52.0 (3.76–19.0)
$C_{av0-8,md}$	μg l^{-1}	–	3.39/65.7 (1.48–8.38)	9	4.01/102 (1.02–12.3)	9	12.2/48.3 (5.64–29.0)
$C_{av0-8,md/D}$	l^{-1}		0.00452/65.7 (0.00197–0.0112)	9	0.00535/102 (0.00136–0.0164)	9	0.00408/48.3 (0.00188–0.00968)
$t_{1/2,md}$	h	7	67.13/20.8 (44.1–82.0)	7	59.8/37.3 (37.9–100)	9	64.9706/15.93 (49.6–82.7)
R_AAUC_{0-8}		–	n.c.	6	179/114 (53.4–656)	7	228/171 (60.7–840)
R_AC_{max}		9	24.0/109 (4.15–76.8)	6	59.7/116 (18.3–192)	7	68.3/124 (22.2–222)

Please note that in A, differences in *n* for different pharmacokinetic parameters after a single dose are explained by lag time. In B, parameters are additionally characterized by an abbreviation for multiple dose (md), to differentiate these parameters from those calculated for first dose.
AUC, area under the concentration–time curve, determined over 8 h (AUC_{0-8}), determined over 8 h and normalized for dose ($AUC_{0-8md/D}$), and determined over 12 h (AUC_{0-12}); bid, twice daily; $C_{av0-\tau}$, average concentration determined over the dosing interval τ of 8 h (C_{av0-8}) or 12 h (C_{av0-12}) and normalized for dose ($C_{av0-\tau/D}$); C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; CV, coefficient of variation; n.c., not calculated; R_AC_{max}, accumulation ratio based on C_{max}; R_AAUC_{0-8}, accumulation ratio based on AUC measured up to 8 hours; tid, thrice daily; $t_{1/2}$, half-life; t_{max}, time to maximum concentration
[a]Median (range)

strongly influenced by the lag time before plasma concentrations increased above the LLOQ after first dosing. Hence, during a significant part of the i.e. the AUC during a dosage interval ($AUC_{0-\tau}$) after the first dose, concentrations were below the LLOQ, resulting in lower AUC values and, consequently, higher ratios. With respect to C_{max}, the observed t_{max} in Cohort 1 was at a median of 24 h. Thus, the C_{max} observed within the dosing interval of the first dose in the multiple-dose groups does not reflect the true C_{max} after a single dose. This, again, results in very high calculated R_A values. These considerations do not change the conclusion that there was significant accumulation, which can be seen from the concentration–time profiles (Figure 5).

Geometric mean and individual concentration–time curves showed several peaks after the last dose of regorafenib on the morning of day 14. These peaks occurred about 5 h and 12 h after administration to the eye, i.e. roughly coinciding with meals. An additional peak was observed on the morning of day 15, i.e. 24 h after the last application. These peaks were probably due to the enterohepatic recirculation of regorafenib. For individual subjects, the time to reach maximum plasma concentration following multiple drug administration ($t_{max,md}$) ranged between 0 h and 12 h after the last dose (Figure 5).

At high interindividual variability (see also Figure 6), systemic regorafenib exposure increased dose dependently after daily doses of regorafenib of 1.5, 2.25 and 9.0 mg. Average concentrations over the 8h dosing interval ($C_{av0-8md}$) were 4.01 μg l^{-1} and 12.2 μg l^{-1} in Cohorts 3 and 4, respectively. Average concentrations over the 12h dosing interval ($C_{av0-12md}$) after regorafenib 0.75 mg bid was 3.39 μg l^{-1}.

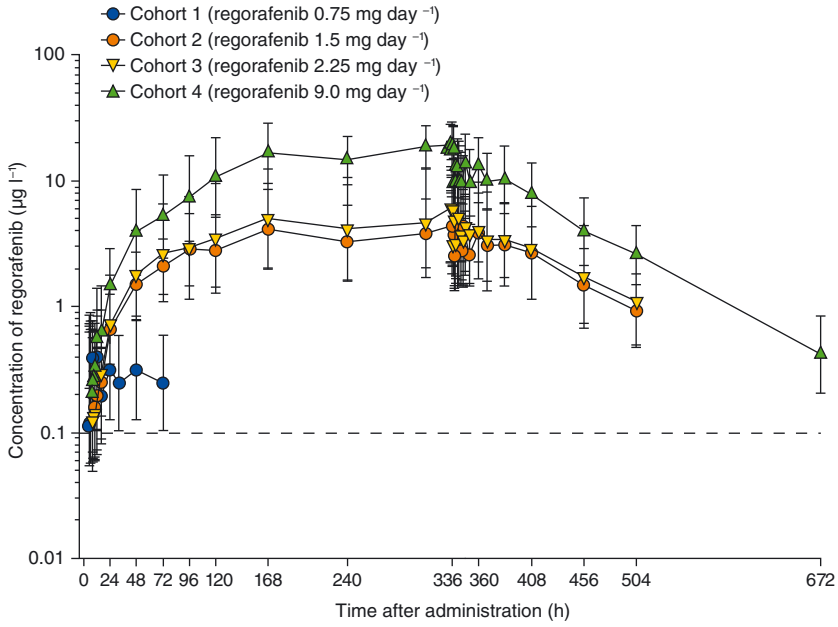

Figure 4

Regorafenib plasma concentrations (µg l^{-1}) after administration of eye drops (0.75 mg day^{-1} single dose; 1.5 mg day^{-1}, 2.25 mg day^{-1} and 9.0 mg day^{-1} multiple dose over 14 days). Geometric means (standard deviation) are shown (semi-logarithmic). Groups sizes were n = 12 (placebo) and n = 36 (regorafenib). Dashed line indicates lower limit of quantification (0.1 µgl^{-1})

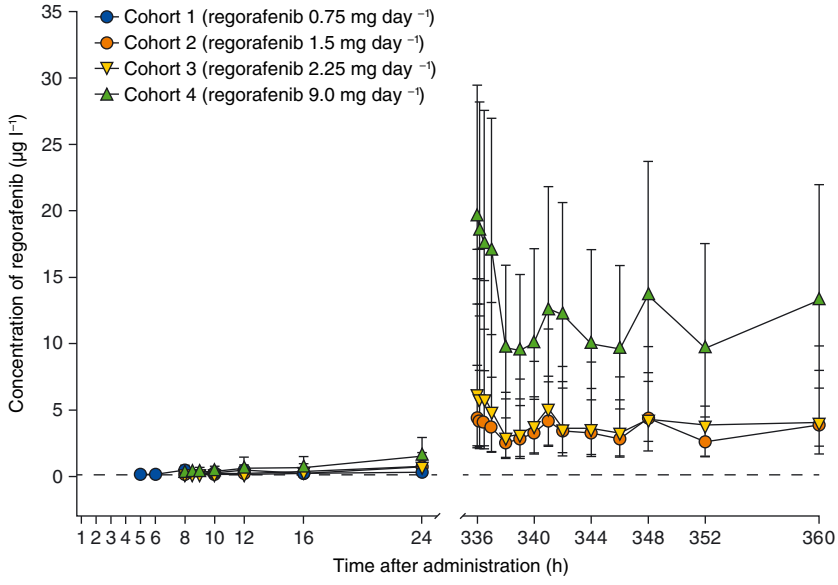

Figure 5

Regorafenib plasma concentrations (µg l^{-1}) on day 0 and day 14 up to 24 h after administration of eye drops (0.75 mg day^{-1} single dose; 1.5 mg day^{-1}, 2.25 mg day^{-1} and 9.0 mg day^{-1} multiple dose over 14 days). Geometric means (standard deviation) are shown (linear scale). Dashed line indicates lower limit of quantification (0.1 µg l^{-1}). Groups sizes were n = 12 (placebo) and n = 36 (regorafenib)

Dose- and body weight-normalized main pharmacokinetic parameters were submitted to an ANOVA, which revealed no statistically significant differences between regorafenib doses over the range 0.75 to 9.0 mg day^{-1} after single and multiple dosing for any of the parameters tested.

Discussion

The present study provided the first-in-human information on general safety, ocular safety, tolerability and convenience, as well as data on systemic exposure after topical

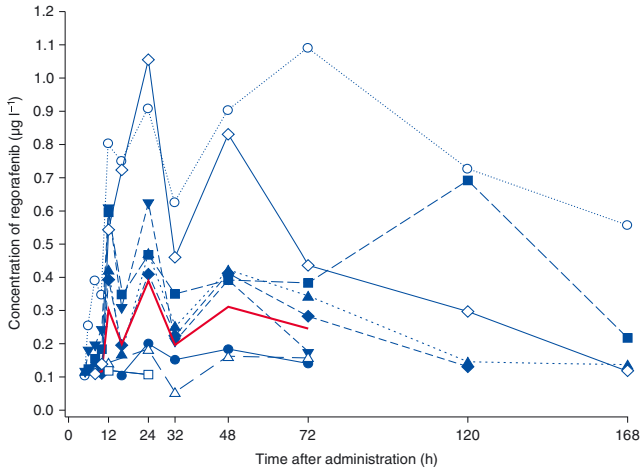

Figure 6

Individual (blue lines) and geometric mean (red line) regorafenib plasma concentrations (µg l^{-1}) after administration of regorafenib eye drops 0.75 mg single dose (Cohort 1) (linear scale). Lower limit of quantification (LLOQ): 0.1 µg l^{-1}. The value below LLOQ at 32 h was derived by substituting this data point below LLOQ by one half of this limit. Symbols refer to different individuals

administration, of single and multiple doses of regorafenib eye drops in healthy male volunteers. The study was conducted in healthy volunteers to allow the extensive monitoring of safety parameters and pharmacokinetics with this unprecedented route of administration for regorafenib, which would not have been possible in a representative (i.e. elderly) patient population.

Regorafenib eye drops were safe and well tolerated at the dose range studied over the 14-day treatment period – i.e. with daily doses up to 9 mg as an eye drop formulation containing 30 mg ml^{-1} regorafenib. No clinically relevant findings related to systemic safety were reported. No local or systemic findings required dose escalation to be stopped or a change to a lower dose. In general, these results confirmed the expectations from preclinical studies in rats and monkeys, which showed that topical administration up to a maximum total dose of 9.6 mg day^{-1} (monkeys) using a concentration of up to 40 mg ml^{-1} for 4 weeks did not reveal any tolerability issues (Joussen *et al.*, manuscript in preparation). However, the first administration of a new drug or formulation in humans required suitable procedures to minimize the risks for the volunteers and to ensure their safety. Therefore, the clinical programme of regorafenib eye drops started with increasing single/multiple topical doses to a minimum number of well-selected young healthy male volunteers. The recommended safe starting dose was deduced on the basis of the no-observed-adverse-effect level (NOAEL) determined in pre-clinical studies, which is considered to be the maximum tested dose of 4.8 mg day^{-1} per eye, or 9.6 mg day^{-1}. Applying a safety factor of 5 (i.e. lower than the default safety factor of 10) was considered appropriate as the pharmacokinetics of regorafenib had previously been fully characterized after oral administration producing high systemic exposure and the eye not being a target organ of observed toxicity. No ill effects were observed in the repeat-dose studies in monkeys, with very sensitive safety measures (such as electroretinography), and a maximum recommended safe starting dose of 1.0 mg day^{-1} per eye for humans was derived. In addition, the next higher dose of regorafenib eye drops given (i.e. 1.5 mg day^{-1} per eye) and the subsequent dose steps to a maximum of 4.5 mg day^{-1} per eye (and 9.0 mg day^{-1}) were only to be implemented if the preceding dose level was deemed by the expert medical team to have been tolerated.

The predominant symptoms reported were solicited reports of blurred vision with transient loss of visual acuity, which was most likely due to the galenic formulation. This finding was not completely unexpected; however, it was important to determine the degree and duration of the functional impairment. The duration of the blurred vision ranged from a few minutes up to 2 h after administration in most of the subjects and resolved without further intervention. With the short-term assessment of visual acuity, a significant drop in the ETDRS test results was seen occasionally, including a short-term decrease of more than 10 letters in single subjects. This finding constituted a formal stop criterion, as predefined by the study protocol, and triggered a written assessment by the expert medical team, stating that this effect was of limited duration and reversible without a need to take any specific measure as it also appeared after placebo. It is possible that the vehicle (in both active and placebo treatments) could have affected the lipid composition of any tears present, resulting in the visual changes. Previous studies have shown that changes in the lipid composition of tears can result in a reduction in visual performance [20]. Based on this interpretation and the lack of any structural ocular findings, it was decided to continue dosing in subjects with intermittent reductions in the ETDRS test results. Remarkably, convenience of treatment was mostly rated as highly acceptable by the volunteers. In addition, as patients with nAMD experience continuous reductions in their visual performance, an intermittent reduction in vision is subjectively much less debilitating than in healthy subjects, and would probably not generate any compliance issues in such patients. These expectations were supported by clinical findings in the phase II study (Joussen *et al.*, manuscript in preparation).

Regorafenib is approved for metastatic CRC and unresectable or metastatic GIST at daily oral doses of 160 mg [16]. At daily oral doses of 60 mg and higher, the incidence of systemic AEs, such as fatigue, voice changes, dermal and gastrointestinal AEs and hypertension rises. As the systemic bioavailability after topical administration in humans was not known prior to the study, these side effects were of special interest in the present study. In addition, dermal AEs (e.g. hand–foot syndrome and rash) are well-known side effects following oral administration of multikinase inhibitors, including regorafenib [21]. As local eye tissues have a particularly high exposure to regorafenib eye drops, the conjunctivae, the conjunctival sac, the nasolacrimal duct and also the nasopharynx were monitored carefully. In the current study, one skin reaction (mild periorbital contact dermatitis) in 48 subjects was reported. The reaction was clearly different from the typical dermal AEs of multikinase inhibitors. Finally, the outcome of this phase I study provided the basis for the dose selection in phase II – i.e. tid administration of 0.75 mg, representing the highest dose administered to one eye, with single drop application.

Elucidation of the effects of systemic exposure after application of the eye drops under conditions of 100% compliance (due to administration by the investigator at the study site) was considered important in order to evaluate the risk of systemic AEs. The pharmacokinetics were therefore characterized thoroughly. The bioavailability of regorafenib eye drops 0.75 mg was 9–15% of that after oral administration. Despite the huge accumulation from single to multiple dosing, the absolute systemic exposure to regorafenib eye drops after multiple 0.75 mg bid or tid administration [as characterized by the AUC over 24 hours after the last dose at steady state (AUC_{0-24ss}) and the maximum concentration at steady state ($C_{max,ss}$)] was 0.15% of that after administration of regorafenib 160 mg orally (600–700-fold difference), 1.5% (AUC) and 1–1.3% (C_{max}) of that after regorafenib 10 mg orally (60–70-fold/80–100-fold difference, respectively) and about 3000-fold lower than the systemic exposure at the NOAEL in dogs. It is therefore considered unlikely that there will be any systemic AEs associated with systemic VEGF inhibition after administration of regorafenib eye drops 0.75 mg bid or tid, and, indeed, none were observed in the present study. Systemic exposure was characterized by a high interindividual variability, with coefficients of variation after multiple dosing generally between 50% and 100%. High interindividual variability has also been found after oral administration of regorafenib and this is probably exacerbated by the even lower relative bioavailability after topical administration.

Although the study design did not allow an intraindividual comparison of the AUC from zero to infinity following a single dose with the $AUC_{0-\tau}$ after multiple doses, which would have allowed the calculation of the linearity factor R_{Lin} and, based on that, an assessment of pharmacokinetic linearity over time, a similar estimation could be done when $AUC_{0-tlast}$ in Cohort 1 was compared with $AUC_{0-\tau}$ in Cohorts 2 to 4. In particular, the almost complete match between the $AUC_{0-tlast}$ of 39.2 μg l^{-1}·h in Cohort 1 and the AUC_{0-12md} of 40.7 μg l^{-1}·h in Cohort 2 provides support for the assumption that there are no changes in pharmacokinetics over time following administration of regorafenib eye drops. The apparent terminal elimination $t_{1/2}$ (60–67 h) after administration of regorafenib eye drops was considerably longer than after oral administration (20–30 h) [16], indicating that the release rate of the drug from the site of administration was probably the rate-limiting step for elimination. The apparent terminal $t_{1/2}$ was probably dominated by the release rate from a drug depot and influenced by enterohepatic recirculation of regorafenib. As melanin may be a depot for drugs administered in the eye [22], and brown eyes have a higher melanin content compared with other eye colours [23], a *post hoc* analysis was undertaken to explore whether there was any relationship between eye colour and systemic pharmacokinetics. Eye colour (brown *vs*. other) was not related to systemic pharmacokinetics. Preclinical data in various animal models (rat, rabbit, monkey) indicate a prolonged persistence of regorafenib in different compartments of the eye (e.g. vitreous, cornea, back of the eye). In these compartments, concentrations were relevantly higher than in the plasma, pointing towards intraocular depots after topical administration as eye drops and absorption-limited systemic pharmacokinetics. However, eye-to-plasma concentration ratios differed between the species examined, so it was not possible to make a prediction for humans. Similarly, concentrations at the target tissue (i.e. the eye) could not be investigated in this clinical phase I study.

The consecutive phase IIa/b study was terminated after completion of phase IIa, for lack of efficacy (NCT02222207; Joussen *et al*., manuscript in preparation], provoking the question of whether the dose in phase I had been selected appropriately. This dose selection had been guided primarily by the human dose prediction derived from the animal studies performed prior to the clinical programme. However, the main outcome measures had been safety and tolerability, as no clinical effects could be obtained from healthy volunteers, and concentrations could not be measured in the target tissues. Therefore, the phase I data did not de-risk the dosing considerations for the later development phases.

In conclusion, regorafenib eye drops were well tolerated over the dose range from 0.75 mg as a single dose (one eye) to 1.5 mg tid (in both eyes) over 14 days. The absolute systemic exposure to regorafenib after multiple administrations of regorafenib eye drops 0.75 mg (one drop bid or tid, one eye treated) was 600–700-fold lower than after oral administration of 160 mg, the approved dose for use in CRC/GIST. The results also confirmed the safety and tolerability profile of the regorafenib eye drop (30 mg ml^{-1}) formulation over the 14-day treatment period.

Competing Interests

F.D. is an employee of SocraTec R&D GmbH. B.S. is an owner and Managing Director of SocraTec R&D GmbH. This research and publication were funded by Bayer AG, Germany (sponsor of the studies and owner of the compound). T.Z., J.H., M.B., M.K.B. and B.R. are employees of Bayer AG. All authors have approved the manuscript as written. The authors declare that they have no other real or potential conflicts of interest.

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Contributors

T.Z., F.D., K.S.K., B.R., B.S. and J.H. were responsible for the study preparation, including study design. F.D. and K.S.K. were responsible for the conduct of the phase I study. F.D., K.S.K. and T.Z. carried out the safety evaluation. J.H. carried out the pharmacokinetic studies, including analysis and interpretation. T.Z., J.H., M.K.B. and B.R. carried out the phase I study, including analysis and interpretation. M.B. was responsible for the clinical statistics. All authors were involved in the writing and review of the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

http://onlinelibrary.wiley.com/doi/10.1111/bcp.13502/suppinfo

Appendix S1 Individual stop criteria