# RESEARCH PAPER

# Safety, pharmacokinetics and pharmacodynamics of the anti-hepcidin Spiegelmer lexaptepid pegol in healthy subjects

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## BACKGROUND AND PURPOSE

Anaemia of chronic disease is characterized by impaired erythropoiesis due to functional iron deficiency, often caused by excessive hepcidin. Lexaptepid pegol, a pegylated structured L-oligoribonucleotide, binds and inactivates hepcidin.

## EXPERIMENTAL APPROACH

We conducted a placebo-controlled study on the safety, pharmacokinetics and pharmacodynamics of lexaptepid after single and repeated i.v. and s.c. administration to 64 healthy subjects at doses from 0.3 to 4.8 mg·kg$^{-1}$.

## KEY RESULTS

After treatment with lexaptepid, serum iron concentration and transferrin increased dose-dependently. Iron increased from approximately 20 μmol·L$^{-1}$ at baseline by 67% at 8 h after i.v. infusion of 1.2 mg·kg$^{-1}$ lexaptepid. The pharmacokinetics showed dose-proportional increases in peak plasma concentrations and moderately over-proportional increases in systemic exposure. Lexaptepid had no effect on hepcidin production or anti-drug antibodies. Treatment with lexaptepid was generally safe and well tolerated, with mild and transient transaminase increases at doses ≥2.4 mg·kg$^{-1}$ and with local injection site reactions after s.c. but not after i.v. administration.

## CONCLUSIONS AND IMPLICATIONS

Lexaptepid pegol inhibited hepcidin and dose-dependently raised serum iron and transferrin saturation. The compound is being further developed to treat anaemia of chronic disease.

## Abbreviations

ACD, anaemia of chronic disease; AUC$_{0-tz}$, area under the plasma concentration–time curve to the last observed concentration; CL, total body clearance of drug from plasma; CL$_R$, renal clearance; $C_{max}$, maximum plasma concentration; PEG, polyethylene glycol; PK, pharmacokinetics; $t_{1/2,ini}$, initial elimination half-life; $t_{1/2}$, terminal elimination half-life; $V_{ss}$, apparent volume of distribution at steady state

## Tables of Links

| TARGETS | |
|---|---|
| **Transporters** | |
| Ferroportin, SLC40A1 | |

| LIGANDS | |
|---|---|
| Hepcidin | IL-12 |
| IL-6 | Tocilizumab |

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

## Introduction

Hepcidin, a 25-amino-acid peptide produced by the liver (Krause *et al.*, 2000; Park *et al.*, 2001; Pigeon *et al.*, 2001), has emerged as the key regulator of systemic iron homeostasis (Ganz, 2003; Roy *et al.*, 2007). It inhibits the release of intracellular iron from duodenal enterocytes, hepatocytes and reticuloendothelial cells by binding to the membrane iron exporter ferroportin, which leads to its internalization and inactivation (Nemeth *et al.*, 2004; Nemeth and Ganz, 2006). Hepcidin is degraded intracellularly after binding to ferroportin (Preza *et al.*, 2013), while fractional urinary excretion is low (Swinkels *et al.*, 2008). Hepcidin expression is induced by circulating iron, body iron stores and by inflammation where it is thought to contribute to host defence. Hepcidin expression is inhibited by erythropoietic activity through certain factors such as growth differentiation factor 15 (Tanno *et al.*, 2007), twisted gastrulation protein homologue 1 (Tanno *et al.*, 2009) and erythroferrone (Kautz *et al.*, 2014). Anaemia of chronic disease (ACD) is attributed to high circulating hepcidin concentrations that result in iron sequestration in reticuloendothelial cells such as macrophages and leads to iron-restricted erythropoiesis in concert with shortened survival of red blood cells and suppression of erythropoiesis by inflammatory cytokines (Weiss and Goodnough, 2005). Currently, the treatment of ACD is difficult: many patients do not respond to erythropoiesis-stimulating agents (MacDougall and Cooper, 2002; Hörl, 2007), while repeated administration of i.v. iron may result in iron overload (Rostoker *et al.*, 2012). By targeting hepcidin, a central pathophysiological process in ACD is addressed directly. Such pharmacological approaches might be more effective and safer than conventional treatments of ACD with erythropoiesis-stimulating agents and i.v. iron.

Lexaptepid pegol (lexaptepid, laboratory code NOX-H94) is a pegylated structured mirror-image oligoribonucleotide (L-RNA), the so-called Spiegelmer® (Spiegelmer is a registered trademark of NOXXON Pharma AG), that binds to human hepcidin with high affinity ($K_D = 0.65 \pm 0.06$ nmol·L$^{-1}$) and thereby blocks its biological function as confirmed *in vitro* and *in vivo* (Schwoebel *et al.*, 2013). At the 5′-end, the L-oligoribonucleotide terminates in a linker to which a branched 40 kDa monomethoxy polyethylene glycol (PEG) unit is covalently attached.

Here, we present the results of the first-in-human study with lexaptepid pegol. This study was conducted in 64 healthy subjects to assess the safety, tolerability and pharmacokinetics of single and repeated i.v. and s.c. doses of lexaptepid and to identify a dosage regimen suitable for clinical trials in patients with ACD.

## Methods

### Subjects

The main selection criteria included healthy men and women of non-childbearing potential aged 18–65 years with a body mass index from 18 to 30 kg·m$^{-2}$ and normal C-reactive protein, clotting, haemoglobin, red cell indices, iron, transferrin saturation, and ferritin.

### Study design

This was a randomized, placebo-controlled, double-blind study of single and repeated doses in healthy volunteers. It was carried out by Hammersmith Medicines Research, London, UK, after approval by the UK Medicines and Healthcare Products Regulatory Agency and the Health and Social Care Research Ethics Committee 1, Lisburn, Northern Ireland. The study was conducted in accordance with good clinical practice, the Declaration of Helsinki from October 1996 and the European Medicines Agency guidelines on strategies to identify and mitigate risks for first-in-human clinical trials (EMA, 2007). All subjects provided written informed consent before participation. This trial was registered under NCT01372137 at clinicaltrials.gov.

In the first part of the study, escalating single i.v. doses of lexaptepid (0.3, 0.6, 1.2, 2.4 or 4.8 mg·kg$^{-1}$ body weight) were infused over 15 min in a citrate buffer containing sucrose to five groups of eight men or women randomized to lexaptepid ($n = 6$) or the corresponding volume of 5% glucose as placebo control ($n = 2$). Dosing was staggered at each dose level: two subjects (one lexaptepid and one placebo) were initially dosed and, 48 h later, another two subjects were dosed. After another 48 h, the remaining four subjects were dosed on the same day. Safety data were reviewed after each step of the staggered dosing and before dose escalation. Pharmacokinetic (PK) and available pharmacodynamic data were also reviewed before dose escalation.

In the second part of the study, escalating repeated i.v. doses of lexaptepid (five doses of 0.6 or 1.2 mg·kg$^{-1}$) were infused over 15 min on alternate days to two groups of

eight men randomized to lexaptepid ($n = 6$) or matching placebo ($n = 2$).

In the final part of the study, repeated doses of 36.5 mg lexaptepid were injected s.c. in a single cohort of eight healthy men (randomized 6:2 to receive lexaptepid or placebo). An initial s.c. dose was followed after 1 week by seven additional doses of lexaptepid on alternate days.

The follow-up period was at least 4 weeks for all cohorts of subjects, and immunogenicity testing was carried out for up to 3 months.

### Safety assessments

Safety assessments were performed on admission to the clinical unit, before dosing and at scheduled intervals after dosing. They included monitoring for adverse events; physical examination; vital signs; clinical laboratory tests with full blood counts and standard biochemistry variables, prothrombin time, international normalized ratio, activated partial thromboplastin time, fibrinogen; 12-lead ECG; and local tolerability at injection sites. Blood concentrations of IL-6 and IL-12 were also monitored after dosing. The single-dose escalation part also included twin-channel cardiac telemetry.

### Pharmacokinetic assessments

Venous blood and urine samples were collected for lexaptepid assay. Blood samples were collected before ($t = 0$) and at frequent intervals for up to 4 weeks after single and repeated i.v. and s.c. dosing. A 24 h urine collection was started immediately after dosing. Concentrations of lexaptepid in plasma and urine were assayed using a quantitative sandwich hybridization assay (Supporting Information) that detects full-length oligonucleotides only, and does not differentiate between lexaptepid with and without bound hepcidin.

Pharmacokinetic parameters were derived by non-compartmental methods using WINNONLIN PROFESSIONAL version 6.2.1 software (Pharsight Corporation, St. Louis, MO, USA). Parameters comprised maximum observed plasma concentration ($C_{max}$), the corresponding time to maximum concentration ($t_{max}$), area under the plasma concentration–time curve to the last observed concentration ($AUC_{0-tz}$), terminal elimination half-life ($t_{1/2}$), apparent volume of distribution at steady state ($V_{ss}$), total body clearance of drug from plasma (CL) and renal clearance ($CL_R$). The initial elimination half-life ($t_{1/2,ini}$) was calculated using a curve-stripping method.

### Pharmacodynamic assessments

Plasma hepcidin concentrations were assayed by Hepcidinanalysis.com (Nijmegen, The Netherlands) as hepcidin-25 in samples taken at the same time points as the PK samples, using a combination of weak cation exchange chromatography and time-of-flight MS, with a hepcidin analogue as an internal standard (Kroot *et al.*, 2010; supplement to Schwoebel *et al.*, 2013). As with the lexaptepid assay, the hepcidin assay does not differentiate between free hepcidin and hepcidin bound to lexaptepid.

The following pharmacodynamic variables were also measured before ($t = 0$) and up to 28 days after dosing: reticulocyte

Hb content (ADVIA 120, Siemens Healthcare, Frimley, UK), serum ferritin, transferrin saturation, serum iron, and C-reactive protein.

### Immunogenicity assessments

The presence of antibodies to lexaptepid in serum samples was assessed by a surface plasmon resonance method established at Eurofins Pharma Bioanalysis Services UK Limited (Abingdon, UK). The method is described in the Supporting Information.

### Statistical methods

This was a descriptive proof-of-principle study, so no formal calculation of sample size was performed. A statistician provided the randomization schedule. Safety and tolerability data were summarized using descriptive statistics, and were not subjected to formal analysis. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) on a Windows XP personal computer. A power model was used to investigate the dose proportionality of $AUC_{0-tz}$ and $C_{max}$. This model is defined as: $Y = a \cdot (dose)^b$. After logarithmic transformation, the power model was formulated as a linear model: $\ln(Y) = b \cdot \ln(dose) + error$. The slope of the regression line ($b$) is 1 for dose proportionality and 0 for dose independence. Descriptive statistics were presented for the pharmacodynamic variables by dose group, including one pooled placebo group.

## Results

### Subjects

Of 64 healthy subjects (45 men and 19 women) overall, 40 were assigned to five subsequent groups of eight patients randomized to single i.v. infusions ($n = 6$) or placebo ($n = 2$) escalated from 0.3 through 0.6, 1.2, 2.4 and 4.8 mg·kg$^{-1}$ lexaptepid. Another two groups of eight subjects were administered five i.v. doses of either 0.6 or 1.2 mg·kg$^{-1}$ lexaptepid versus placebo on alternate days over 10 days. The eighth group was treated with repeated s.c. injections of 36.5 mg lexaptepid (fixed dose) or placebo, administered as a pilot dose and a week later as seven injections on alternate days over 2 weeks. Baseline demographics were similar across all treatment groups. One subject (2%) withdrew consent from the i.v. 0.6 mg·kg$^{-1}$ repeated-dose group, after receiving four doses of lexaptepid. The remaining 63 subjects (98%) completed the study.

### Safety

Lexaptepid was generally safe and well tolerated. The observed adverse events were mostly minor and transient and resolved spontaneously; their incidence and severity were similar over all treatment groups, with the exception of local injection site reactions after s.c. administration. Two subjects developed a skin rash at the sites of s.c. lexaptepid injections, starting 2 weeks after their final dose. The rash persisted for about 2 months and was treated with a topical steroid. Lymphocyte stimulation tests were negative in one subject and inconclusive in the other, so the exact aetiology of the rashes, be it irritation or sensitization, could not be determined.

Other injection site reactions were injection site haematoma reported by three subjects (6%) after i.v. lexaptepid and by one subject (2%) after s.c. placebo injection. Presumed viral infection (mostly clinical nasopharyngitis: 14 subjects, 22%), headache (12 subjects, 19%), fatigue (6 subjects, 9%), oropharyngeal pain (5 subjects, 8%), dizziness (3 subjects, 5%), palpitation (2 subjects, 3%), dyshidrosis (2 subjects, 3%) and insomnia (2 subjects, 3%) were the only adverse events, whether related and unrelated to treatment, that occurred more than once. A single serious adverse event occurred 54 days after a single i.v. dose of 1.2 mg·kg$^{-1}$ lexaptepid: after the sudden onset of occipital headache, a subject was admitted to hospital to exclude an intracerebral bleed. The adverse event was eventually diagnosed as muscular strain of the neck and deemed unrelated to treatment with lexaptepid. A list of all adverse events occurring after administration of the study medication is provided in the Supporting Information.

Mild and transient increases in liver transaminases (<2× upper limit of normal) in 12 asymptomatic subjects (25%) were observed after single doses of lexaptepid ≥2.4 mg·kg$^{-1}$ and after doses of 1.2 mg·kg$^{-1}$ repeated on alternate days. These transaminase increases were not considered as adverse events. Coagulation, including prothrombin time, international normalized ratio, activated partial thromboplastin time, serum bilirubin, and eosinophil count were unchanged. Lexaptepid was not associated with clinically relevant changes in physical examination, vital signs, ECG, standard clinical laboratory tests or IL-6 and IL-12 results.

## Pharmacokinetics

All six subjects given lexaptepid from each dose group had enough data to allow calculation of reliable estimates of PK parameters and were included in the PK assessments.

The main PK parameters and the concentration–time profiles after single i.v. infusion are summarized in Table 1 and Figure 1. Peak plasma concentrations of lexaptepid were usually recorded within the first hour after dosing (0.25–1 h). Mean $C_{max}$ increased from 0.52 to 9.75 μmol·L$^{-1}$ after single i.v. doses of 0.3–4.8 mg·kg$^{-1}$ lexaptepid respectively. Using a power model after logarithmic transformation, dose proportionality was concluded for $C_{max}$, as the mean slope in the
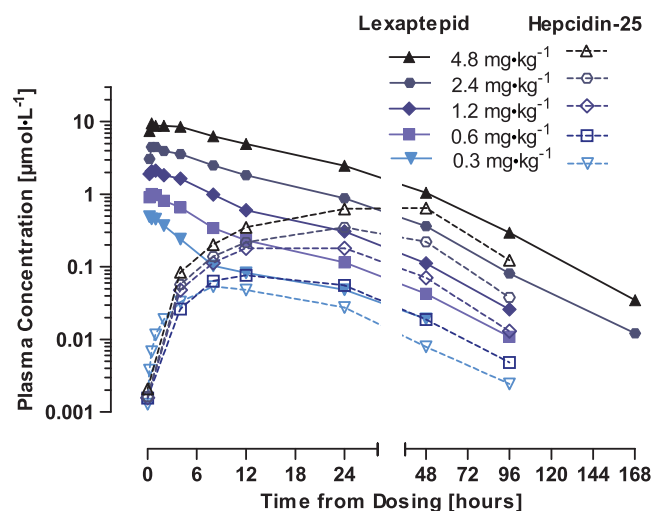


**Figure 1**

Concentration–time profiles of lexaptepid and hepcidin-25 in plasma after single i.v. administration of lexaptepid. Lexaptepid and hepcidin data are expressed as geometric means of six subjects per dose level.

power model analysis was 1.064 (0.991–1.138 μmol·L$^{-1}$, 95% confidence intervals). Supra-proportional increases in the AUC occurred across the same dose range, with a mean slope for $AUC_{0-tz}$ of 1.37 with the 95% confidence interval [1.21–1.49] outside the 0.8–1.25 interval. The dose of lexaptepid increased 16-fold from 0.3 to 4.8 mg·kg$^{-1}$, whereas $AUC_{0-tz}$ increased by a factor of 45.

Systemic plasma clearance (CL) was low and decreased from 293 to 115 mL·h$^{-1}$ as the dose of lexaptepid increased. Renal clearance ($CL_R$) ranged from 5.47 to 7.00 mL·h$^{-1}$ and was low compared with CL. The apparent volume of distribution at steady state ($V_{ss}$) decreased from 5.0 to 3.3 L with increasing dose, but remained within the range of the plasma volume. The lexaptepid plasma concentration–time curve showed a biphasic elimination profile, with an initial rapid decline followed by a slower terminal elimination phase (Figure 1). In some subjects with particularly low hepcidin concentrations, the slower elimination phase was absent (data not shown).

## Table 1

Main pharmacokinetic parameters after single i.v. and s.c. administration of lexaptepid

| Dose/route | $C_{max}$ (μmol·L$^{-1}$) | $AUC_{0-tz}$ (μmol·L$^{-1}$·h$^{-1}$) | $t_{1/2}$ (h) | CL (mL·h$^{-1}$) | $V_{ss}$ (L) |
|---|---|---|---|---|---|
| 0.3 mg·kg$^{-1}$/i.v. | 0.517 (18.2) | 4.71 (76.4) | 14.1 (81.0) | 293 (72.0) | 5.01 (42.7) |
| 0.6 mg·kg$^{-1}$/i.v. | 1.04 (34.1) | 12.2 (44.4) | 15.4 (85.8) | 229 (37.4) | 4.47 (41.4) |
| 1.2 mg·kg$^{-1}$/i.v. | 2.16 (12.0) | 29.8 (22.7) | 22.4 (38.9) | 184 (19.0) | 4.08 (28.3) |
| 2.4 mg·kg$^{-1}$/i.v. | 4.70 (16.0) | 79.3 (14.5) | 23.2 (19.3) | 145 (25.6) | 3.63 (33.5) |
| 4.8 mg·kg$^{-1}$/i.v. | 9.75 (16.1) | 211 (12.4) | 26.1 (12.2) | 115 (21.4) | 3.30 (27.7) |
| 38.5 mg/s.c. | 0.0898 (92.3) | 8.14 (84.2) | 43.0 (57.1) | n.d. | n.d. |

Data shown are geometric means (with geometric coefficient of variation) for $n = 6$ subjects. $C_{max}$, maximum observed lexaptepid plasma concentration; $AUC_{0-tz}$, area under the plasma concentration–time curve to the last observed concentration; $t_{1/2}$, terminal elimination half-life; CL, plasma clearance; $V_{ss}$, volume of distribution at steady state; n.d., not determined.

Rapid decrease in plasma concentrations within the first 8 h after dosing was characterized by a dose-dependent initial half-life ($t_{1/2,ini}$) of 1.8–8.0 h. The terminal half-life ($t_{1/2}$) increased dose-dependently from 14.1 to 26.1 h. Urinary excretion of unchanged full-length lexaptepid during the first 24 h after dosing increased from 1.35 to 3.69% with the dose of lexaptepid.

After repeated i.v. administration of 0.6 or 1.2 mg·kg$^{-1}$ lexaptepid on alternate days (Figure 2), the plasma concentration remained nearly constant, as judged by $C_{max}$ and AUC after first and last doses. Mean trough concentrations determined at 48 h post-dose were similar for all dose administrations at each of the two dose levels (0.09–0.11 µmol·L$^{-1}$ after 0.6 mg·kg$^{-1}$ and 0.14–0.20 µmol·L$^{-1}$ after 1.2 mg·kg$^{-1}$). The PK parameters after repeated i.v. doses of lexaptepid were similar to those after equivalent single i.v. doses; the ratios of AUC at steady state to AUC after a single dose were 0.90 and 1.21 for the 0.6 and 1.2 mg·kg$^{-1}$ dose groups, respectively, with similar results for $C_{max}$.

The concentration–time profile after single and repeated s.c. administration of 36.5 mg lexaptepid (fixed dose) is displayed in Figure 3 together with the corresponding hepcidin concentrations. The mean $C_{max}$ of lexaptepid after the pilot dose was 0.090 µmol·L$^{-1}$ and was reached after 24–51 h. The $t_{1/2}$ after single and repeated s.c. doses was 43 h, which was longer than that after i.v. administration, most likely due to the continued influx of drug from the s.c. depot during the elimination phase. Repeated s.c. injections of 36.5 mg on alternate days resulted in moderate accumulation in plasma, with an accumulation ratio of 2.8 for $C_{max}$. Steady state was reached after three injections.

## Pharmacodynamics

*Hepcidin.* Mean plasma hepcidin concentration–time profiles are presented together with the lexaptepid PK profiles in Figure 3 after single and repeated s.c. administration. Four
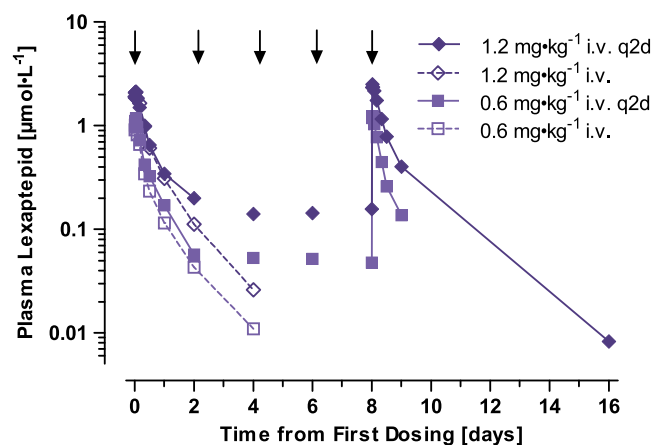


**Figure 3**

Concentration–time profiles of lexaptepid and hepcidin-25 in plasma after single and repeated s.c. administration of lexaptepid. Arrows indicate injection of lexaptepid. Data shown for days 7 to 17 represent pre-dose concentrations. Lexaptepid and hepcidin data are expressed as geometric means of six subjects.

subjects had low hepcidin at baseline and at subsequent time points in comparison with the rest of the study population. Mean plasma concentrations of total hepcidin increased dose-dependently with lexaptepid treatment, without ever exceeding the plasma concentration of lexaptepid. Because the analytical method does not differentiate between free hepcidin and hepcidin bound to lexaptepid, and hepcidin



**Figure 2**

Pharmacokinetic profiles of single and repeated i.v. administration of lexaptepid. Arrows indicate time of lexaptepid administration. After the first and last doses, a complete pharmacokinetic profile was determined; data of days 2, 4 and 6 represent pre-dose concentrations. Data are expressed as geometric means of six subjects per dose level and are compared with data of single dose administration of 0.6 and 1.2 mg·kg$^{-1}$.
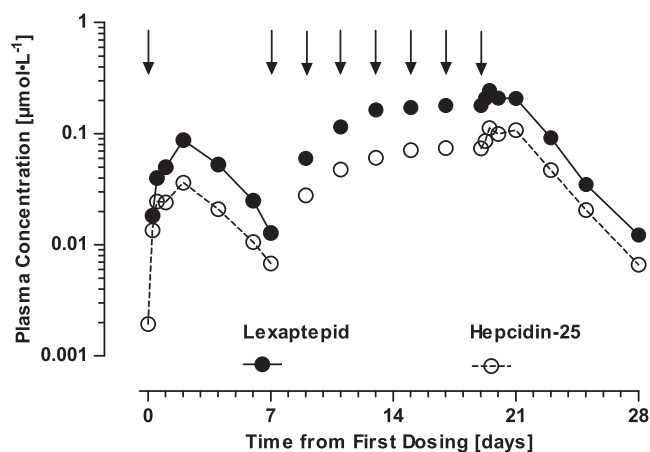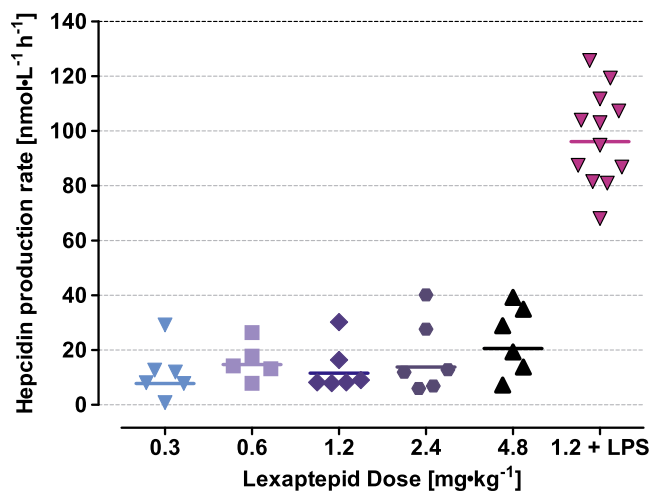


**Figure 4**

Production rates of hepcidin-25 after single i.v. doses of lexaptepid. Hepcidin production rates were calculated based on the linear increase of total plasma hepcidin-25 concentration between pre-dose and 4 h after lexaptepid administration. Individual production rates are presented, with geometric means of six subjects. The 4 h data of one subject in the 0.6 mg·kg$^{-1}$ dose group, with concentration below the limit of quantitation, were excluded. Hepcidin production rates from subjects treated with LPS (van Eijk *et al.*, 2014) are shown for comparison.

## Table 2

AUC for serum iron parameters after single i.v. doses of lexaptepid compared with baseline (means ± SD)

| Dose (mg·kg$^{-1}$) | n | Serum iron AUC (µmol·L$^{-1}$·h$^{-1}$) | TSAT AUC (%·h$^{-1}$) | Serum ferritin AUC (µg·L$^{-1}$·h$^{-1}$) |
|---|---|---|---|---|
| Placebo | 10 | 44.6 ± 28.4 | 62.5 ± 41.0 | 29.5 ± 45.3 |
| 0.3 | 6 | 47.6 ± 51.9 | 82.2 ± 76.9 | 15.5 ± 31.4 |
| 0.6 | 6 | 66.9 ± 40.3 | 111 ± 60.1 | 44.4 ± 53.2 |
| 1.2 | 6 | 193 ± 53.7 | 321 ± 137 | 125 ± 206 |
| 2.4 | 6 | 134 ± 45.0 | 239 ± 97.8 | 371 ± 291 |
| 4.8 | 6 | 225 ± 138 | 422 ± 334 | 644 ± 670 |

Area under the data–time curve above baseline from time 0 to 24 h after the last dose. TSAT, transferrin saturation.

concentration never exceeds the lexaptepid concentration, the hepcidin concentrations measured at later time points represent mainly bound hepcidin. The hepcidin production rate, estimated by the change in total plasma hepcidin concentration over time ($\Delta c\Delta t^{-1}$) between pre-dose and 4 h post-lexaptepid administration, was largely constant over the dose range studied (Figure 4).

*Reticulocyte Hb content.* No change was observed in the reticulocyte Hb content after single and repeated i.v. and s.c. dosing compared with placebo, and at no time point after dosing did it change by more than 30%.

*Iron metabolism variables.* In the single-i.v.-dose groups, lexaptepid up to 0.6 mg·kg$^{-1}$ affected neither serum iron nor transferrin saturation nor serum ferritin. In the repeated-dose group, however, increases of serum iron, transferrin saturation and serum ferritin were observed, even after the first dose of 0.6 mg·kg$^{-1}$ lexaptepid. After single doses of ≥1.2 mg·kg$^{-1}$ and repeated doses of ≥0.6 mg·kg$^{-1}$ lexaptepid, serum iron, transferrin saturation and serum ferritin increased dose-dependently (Table 2 and Figures 5, 6; Supporting Information, Figures S1, S2).
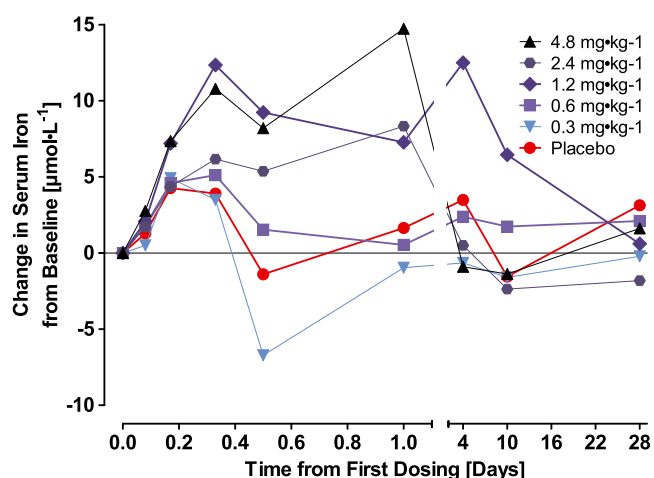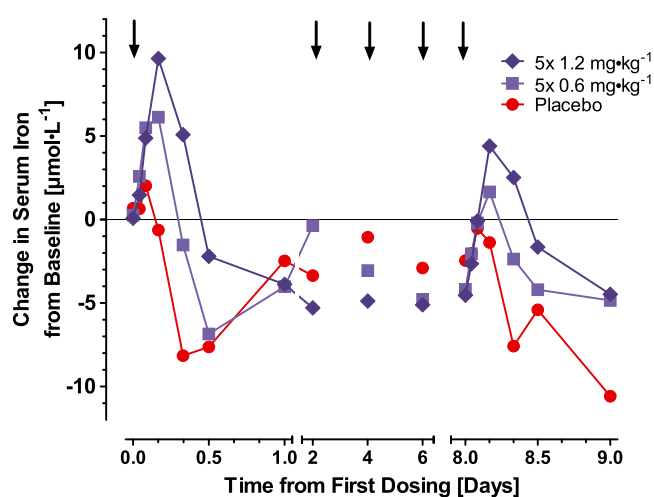
## Figure 6

Mean serum iron concentrations after repeated i.v. doses of lexaptepid. After the first and last doses, a complete profile was determined. Data at days 2, 4 and 6 represent pre-dose concentrations. Arrows indicate lexaptepid administration.

Transferrin saturation rose above 80% in two subjects, one subject each treated with 1.2 and 4.8 mg·kg$^{-1}$ with peak transferrin saturation of 83.3 and 80.8%.

## Immunogenicity

No antibodies specific to the oligonucleotide chain of lexaptepid were detected at any point during the study. Antibodies specific to the PEG moiety were detected in pre-dose samples with high inter-individual variability. After treatment with lexaptepid, anti-drug antibody responses increased above the calculated cut point of +20.5% in 10 subjects (21.3%) after lexaptepid in a total of 18 samples (16.8%) and in four subjects (25%) after placebo in a total of four samples (11.1%). The lack of difference in the number of positive samples between the individual dose groups, irrespective of dose strength, dosing schedule or dosing route, suggests that those effects were not related to treatment with lexaptepid. The presence of anti-PEG antibodies did not affect the pharmacodynamic effect on serum iron or the PK profile of lexaptepid, as supported by the lack of correlation

## Figure 5

Serum iron profiles after single i.v. doses of lexaptepid.

between pre-existing or induced antibodies and the rate of lexaptepid plasma elimination (data not shown).

## Discussion and conclusions

This first-in-human study with lexaptepid showed that it was safe and generally well tolerated: The main safety findings after i.v. administration were mild and transient increases in liver transaminases, which were observed when single lexaptepid doses were $\geq 2.4$ mg·kg$^{-1}$, or repeated doses were 1.2 mg kg$^{-1}$ on alternate days, corresponding to a weekly dose of 4.2 mg kg$^{-1}$. i.v. treatment with lexaptepid had no effect on adverse events compared with placebo. After s.c. administration, two subjects developed local reactions, after a 2-week interval, related to lexaptepid treatment. At present, the contribution of lexaptepid itself or the sucrose-containing formulation to these dermal reactions is unclear.

The pharmacokinetics of lexaptepid after short i.v. infusion over 15 min were characterized by a dose-proportional increase in maximum plasma concentrations, observed within 1 h after administration, and a slightly supraproportional increase in systemic exposure over the dose range studied. After s.c. administration, peak concentrations were significantly delayed until between 24 and 48 h after injection. Lexaptepid did not accumulate after repeated i.v. infusions of 0.6–1.2 mg·kg$^{-1}$, but moderate accumulation occurred after repeated s.c. injection of lexaptepid. Steady state was reached after three of the eight alternate daily s.c. injections of 36.5 mg. A mean $C_{max}$ of about 0.3 μmol·L$^{-1}$ was attained at 12 h after the final dose. The apparent volume of distribution was in the range of 3–5 L, corresponding closely to the intravascular plasma volume. Plasma clearance of lexaptepid was low ($<300$ mL·h$^{-1}$) and seemed to decrease further with increasing lexaptepid dose. The $CL_R$ calculated from excretion of unchanged Spiegelmer in urine did not exceed 7 mL·h$^{-1}$. This represented a renally eliminated fraction of less than 4% over the first 24 h after dosing. The discrepancy between CL and $CL_R$ points to an alternative route of elimination. In cynomolgus monkeys, vacuolated macrophages, presumably containing the pegylated Spiegelmer, were identified by histopathological examination in various organs, including the liver and spleen (data not shown). The elimination was mostly biphasic, with a fast $t_{1/2,ini}$ and a $t_{1/2}$ of approximately 24 h. The absence of the slower elimination phase in some subjects with low hepcidin concentrations may indicate that the elimination of lexaptepid is slower once it binds hepcidin. The $t_{1/2}$ at lower doses may have been underestimated due to the shorter time to the last measurable drug concentration in the lower-dose groups, which was 48–96 h in the 0.3 mg·kg$^{-1}$ dose group, compared with 240 h in the 4.8 mg·kg$^{-1}$ dose group due to the limited sensitivity of the PK assay. As a result, the computation of $t_{1/2}$ using at least four points of the terminal PK curve resulted in the interference by the faster $t_{1/2,ini}$ in the lower-dose groups and hence the underestimation of $t_{1/2}$ and possibly also of the systemic exposure.

After administration of lexaptepid, total hepcidin increased in plasma. This increase represented hepcidin bound to the Spiegelmer and protected from plasma elimination. Peak concentrations of total hepcidin increased dose-dependently, but hepcidin never exceeded the concentration of lexaptepid on a molar basis. Until approaching saturation of the binding capacity of lexaptepid, the increase in the hepcidin concentration was linear (defined as 'production rate') and independent of the dose of lexaptepid. Similar dose-dependent changes in total hepcidin concentrations, that is, constant increase until saturation of the binding capacity and dose-dependent peak concentration, were observed in cynomolgus monkeys treated with the anti-hepcidin antibody Ab 12B9m (Xiao et al., 2010). These findings suggest that hepcidin production is not induced by lexaptepid and that hepcidin is efficiently captured by lexaptepid, as further supported by the pharmacodynamic effects on iron variables. In contrast, after endotoxin-induced inflammation in healthy subjects, the increase in serum hepcidin was approximately four times faster, suggestive of a higher hepcidin production rate. Even under these conditions, a single i.v. dose of 1.2 mg·kg$^{-1}$ lexaptepid was able to inactivate the effects of hepcidin (van Eijk et al., 2014).

Serum iron and transferrin saturation increased dose-dependently after i.v. doses of lexaptepid of 0.6 mg·kg$^{-1}$ or higher. Elevations of transferrin saturation above 80% were rare, indicating a low risk of the occurrence of toxic iron species such as non-transferrin-bound iron and labile plasma iron (de Swart, Haematologica, 2016). Increases in iron and transferrin saturation are the expected result of the elevated release of intracellular iron into the circulation by increased ferroportin activity. In the investigated population of healthy subjects with normochromic and normocytic reticulocytes and mature red blood cells, the increased iron availability did not result in an elevation of the haemoglobin load of reticulocytes, as shown by constant reticulocyte haemoglobin. Serum ferritin increased consistently and dose-dependently after single doses of lexaptepid. Under conditions of elevated iron stores in the reticuloendothelial system (RES), the inhibition of hepcidin should release intracellular iron from the RES and thus, over time, should decrease serum ferritin concentrations (Cohen et al., 2010), as observed in a study in cancer patients with ACD and high baseline ferritin who were treated with lexaptepid over a period of 4 weeks (Georgiev et al., 2014). Therefore, serum ferritin increases observed in the present study are not likely to reflect cellular ferritin-bound iron stores. The cause of the increase in serum ferritin would be merely speculation, as despite its long history of use in the assessment of body iron stores, the source and detailed secretory pathway of serum ferritin from cells are not completely understood (Wang et al., 2010).

Currently, no drug acting specifically on the hepcidin–ferroportin axis is approved for routine clinical use. The anti-IL-6 receptor antibody tocilizumab, approved for the treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis, was effective in reducing hepcidin production and elevating haemoglobin in patients with Castleman disease, characterized by high IL-6 (Song et al., 2010). Tocilizumab is, however, unsuitable to treat ACD due to its immunosuppressive effects.

Several drugs are in development to inhibit hepcidin production, to bind and inactivate hepcidin or to inhibit

the interaction between hepcidin and ferroportin, as reviewed by Fung and Nemeth (2013). Among these, an antibody (LY2787106) and an anticalin (PRS-080) targeting hepcidin and an antibody targeting ferroportin (LY2928057) (Witcher *et al.*, 2013) are in clinical development. These compounds have shown the expected pharmacodynamic effects on serum iron in animals. Clinical data from healthy subjects and cancer patients were presented that confirm their ability to increase serum iron concentrations (Moebius *et al.*, 2015; Vadhan-Raj *et al.*, 2015). In addition, with its lower immunogenic potential, the Spiegelmer lexaptepid may have an advantage over antibodies in a chronic treatment setting.

In conclusion, in this first-in-human study, lexaptepid showed a favourable safety, PK and pharmacodynamic profile. Offering a new therapeutic approach, it may complement the armamentarium of anaemia treatment, reduce doses of erythropoiesis-stimulating agents and i.v. iron and improve the safety and efficacy of anaemia treatment. Clinical studies in ACD in cancer patients and in dialysis patients are ongoing and should provide further evidence of the value of the hepcidin–ferroportin axis as a target for treatment of ACD.

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## Author contributions

M.B., S.W. and B.C. conducted the clinical study, interpreted the findings and wrote the manuscript. S.Z. and S.V. planned, performed and interpreted the pharmacokinetics, monitored hepcidin measurements and edited the manuscript. D.W.S. supervised measurements of hepcidin, interpreted findings and edited the manuscript. L.S., F.S. and K.R. designed the study; gathered, cleaned and interpreted the data; and edited the manuscript.

## Conflict of interest

This study was sponsored by NOXXON Pharma AG, Berlin, Germany. M.B., S.W. and B.C. are employees of Hammersmith Medicines Research. S.Z., S.V., L.S., F.S. and K.R. are employees of NOXXON Pharma AG. D.W.S. is involved in the Hepcidinanalysis.com initiative, by which the Radboud University Nijmegen Medical Center offers high-quality hepcidin measurement to the scientific and medical community. The other authors have no conflicts of interest to declare.

## Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organizations engaged with supporting research.

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## Supporting Information

Additional Supporting Information may be found in the on-line version of this article at the publisher's web-site:

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**Figure S1** Serum ferritin: change from baseline after single administration of lexaptepid pegol
**Figure S2** Transferrin saturation: change from baseline after single administration of lexaptepid pegol
**Table S1** Overview of treatment-emergent adverse events