

Iron uptake and ferrokinetics in healthy male subjects of an iron-based oral phosphate binder (SBR759) labeled with the stable isotope ^{58}Fe

Cite this: *Metallomics*, 2014, 6, 2062

Hans-Peter Gschwind,^{*a} Dietmar G. Schmid,^a Friedhelm von Blanckenburg,^{†b} Marcus Oelze,^{‡d} Kirsten van Zuilen,^{‡d} Alan J. Slade,^c Sylvie Stitah,^d Daniel Kaufmann^e and Piet Swart^a

SBR759 is a novel polynuclear iron(III) oxide–hydroxide starch-sucrose-carbonate complex being developed for oral use in chronic kidney disease (CKD) patients with hyperphosphatemia on hemodialysis. SBR759 binds inorganic phosphate released by food uptake and digestion in the gastro-intestinal tract increasing the fecal excretion of phosphate with concomitant reduction of serum phosphate concentrations. Considering the high content of ~20% w/w covalently bound iron in SBR759 and expected chronic administration to patients, absorption of small amounts of iron released from the drug substance could result in potential iron overload and toxicity. In a mechanistic iron uptake study, 12 healthy male subjects (receiving comparable low phosphorus-containing meal typical for CKD patients: ≤ 1000 mg phosphate per day) were treated with 12 g (divided in 3×4 g) of stable ^{58}Fe isotope-labeled SBR759. The ferrokinetics of [^{58}Fe]SBR759-related total iron was followed in blood (over 3 weeks) and in plasma (over 26 hours) by analyzing with high precision the isotope ratios of the natural iron isotopes ^{58}Fe , ^{57}Fe , ^{56}Fe and ^{54}Fe by multi-collector inductively coupled mass spectrometry (MC-ICP-MS). Three weeks following dosing, the subjects cumulatively absorbed on average 7.8 ± 3.2 mg (3.8–13.9 mg) iron corresponding to $0.30 \pm 0.12\%$ (0.15–0.54%) SBR759-related iron which amounts to approx. 5-fold the basal daily iron absorption of 1–2 mg in humans. SBR759 was well-tolerated and there was no serious adverse event and no clinically significant changes in the iron indices hemoglobin, hematocrit, ferritin concentration and transferrin saturation.

Received 30th April 2014,
 Accepted 20th June 2014

DOI: 10.1039/c4mt00126e

www.rsc.org/metallomics

1 Introduction

Serum phosphate homeostasis is primarily regulated by the body's ability to excrete excess dietary phosphate *via* the kidneys. Patients with chronic renal insufficiency, who have varying degrees of renal function, accumulate excess phosphate resulting in a condition known as hyperphosphatemia. First generation, calcium- or aluminum-containing phosphate binders are effective in reducing the absorption of phosphate from the diet, but require large doses and may result in accumulation of calcium

or aluminum. Intense research efforts have led to the development of a second generation of potent, calcium and aluminum-free, phosphate binders such as sevelamer hydrochloride (HCl) (RenaGel[®]), a cross-linked poly-allylamine hydrochloride (Bleyer, 1999)¹ or fosrenol[™], a lanthanum carbonate compound (D'Haese, 2003).² While sevelamer-HCl has proven efficacy it is associated with possible induction of metabolic acidosis and gastrointestinal (GI) side effects (Brezina, 2004).³ In addition, sevelamer-HCl does not bind phosphate efficiently in an acidic environment. Lanthanum carbonate also demonstrates significant phosphate-binding properties and good efficacy but has also been associated with hypercalcemia (Hutchison, 2009)⁴ (Hutchison, 2005)⁵ (D'Haese, 2003).² Studies in both rats (Slatopolsky, 2005)⁶ and humans (Spasovski, 2006)⁷ have suggested that long-term lanthanum administration carries the risk of blood and tissue accumulation.

SBR759 is a novel polynuclear iron(III) oxide–hydroxide-starch-sucrose-carbonate complex designed for oral use to specifically bind with high affinity and excrete inorganic phosphate. SBR759 is an odorless, water-insoluble, slightly sweet tasting powder formulation. The compound was developed with SeBo GmbH, Germany for the treatment of hyperphosphatemia commonly

^a Drug Metabolism & Pharmacokinetics (DMPK)/Integrated Drug Disposition (IDD), Novartis Pharma AG, 4002 Basel, Switzerland. E-mail: hans-peter.gschwind@bluewin.ch

^b Institute for Mineralogy, Leibniz University of Hannover, Germany

^c Novartis Institute for Biomedical Research (NIBR)/Translational Medicine, Novartis Corporation, East Hanover, USA

^d Clinical Science and Innovation, Novartis Pharma AG, Basel, Switzerland

^e Chemical and Analytical Development, Novartis Pharma AG, Basel, Switzerland

[†] Present address: GFZ German Research Center for Geosciences, Helmholtz Centre Potsdam, Germany.

[‡] Present address: Institute of Geological Sciences, University of Berne, Switzerland.



observed in patients with CKD. SBR759 has been shown to be an effective phosphate binder *in vivo* and shows rapid, selective, and high *in vitro* binding of phosphate at both highly acidic and neutral pH with phosphate-binding capacity similar to currently available agents (Hergesell, 1999)^{8a} (Hergesell, 1999).^{8b} SBR759 lowered serum phosphate concentrations rapidly and to a clinically meaningful extent across a wide dose range with good tolerability (Block, 2010).⁹ In phase II dose titration studies assessing Asian patients requiring hemodialysis, SBR759 demonstrated superior efficacy in phosphate control compared with sevelamer-HCl, an approved phosphate binder (Chen, 2011)¹⁰ (Fukagawa, 2014).¹¹

SBR759 is a chemical complex of polymeric nature. It is a novel member of the iron-oxide-hydroxide compound class, which is different from previously described iron-oxide-hydroxides such as goethite, akaganéite or lepidocrocite. Even though it resembles ferrihydrite in its characteristics, it is different to ferrihydrite and known iron-oxides-hydroxides as follows: (i) sucrose, carbonate and very likely starch are included in the coordination sphere of the iron atoms and bound to the iron atoms by a coordinative bond; (ii) Mössbauer spectroscopy showed that SBR759 is different to standard iron(III)-oxides. Thus, it can be described formally as the polynuclear iron(III) oxide-hydroxide-starch-sucrose-carbonate complex and is therefore not a simple mixture of these starting materials. Phosphate binding by SBR759 is accomplished by a ligand exchange mechanism displacing sucrose carbonate or hydroxyl groups, the latter similar to the iron(III) oxide-hydroxide complex described in (Sigg, 1980)¹² forming predominantly binuclear bidentate complexes (Parfitt, 1975).¹³ The iron in SBR759 is covalently bound to the starch-saccharose complex and is very stable against digestion in the human gastro-intestinal tract (GIT). However, due to the high iron content (~20% w/w) of SBR759 and gram quantity doses required for treatment, chronic administration may be accompanied by absorption of iron, released from the iron(III) complex. With chronic treatment often required in patients with chronic kidney disease, absorption of this exogenous iron may result in the potential for iron overload and toxicity. Iron toxicity results when free iron not bound to transferrin (NTBI) appears in the blood, forming labile plasma iron (LPI) and catalyzing the generation of reactive oxygen species (ROS) (Anderson, 2007)¹⁴ that can damage tissues. Likewise, generation of ROS in the gut for prolonged periods of time could lead to epithelial cell damage and may facilitate pathogen entry to the organism (Chávez, 2007).¹⁵ *In vitro* tests have shown that the iron oxides present in SBR759 are not redox-active, a prerequisite for the above mentioned side effects (Novartis, unpublished data).

Nonclinical testing with ⁵⁹Fe-radiolabeled SBR759 in dogs demonstrated that less than 0.2% of the SBR759-related iron dose was absorbed (Novartis, unpublished data). In order to gain insight into the ferrokinetics and to quantify the extent of SBR759-related iron absorption following oral administration of SBR759, a phase I clinical study with stable ⁵⁸Fe isotope-labeled SBR759 in healthy male subjects was performed. The ferrokinetics of [⁵⁸Fe]SBR759-related total iron was followed in whole blood (over 3 weeks) and in plasma (over 26 hours) by analyzing the isotope ratios of the natural iron isotopes by

MC-ICP-MS. Apart from whole blood, plasma was chosen because it comprises a more dynamic compartment concerning the turnover of transferrin-bound iron as compared to red blood cells which are characterized by a 120 day erythrocyte life span in circulation (Walczyk, 2005).¹⁶ Aside from tracing the ferrokinetics and the extent of the oral absorption of [⁵⁸Fe]SBR759-related iron in blood and plasma determined upon changing ⁵⁸Fe/⁵⁶Fe-isotope ratios, a possible difference in the ⁵⁶Fe/⁵⁴Fe isotope signature between erythrocytes (whole blood) and plasma was measured by MC-ICP-MS in samples collected between 0 and 26 hours post first SBR759 dose. These results are presented in a companion paper that also contains the measured iron concentrations and iron isotope ratio data used for the present study in an Online Supplement S4 (von Blanckenburg, submitted).¹⁷ This study also provided insight into the applicability of the MC-ICP-MS technique in measuring isotope-labeled iron in human blood and plasma.

2 Materials and methods

2.1 Selecting iron isotopes to investigate iron uptake in human

Biomarkers such as transferrin concentration and ferritin saturation can be used as clinical indicators as well as the state of iron stores including iron overload. Direct quantification of ferrokinetics was typically obtained through the use of isotope labeled iron compounds. Applying radioactive iron isotopes such as ⁵⁹Fe is a very sensitive and a specific approach, but it presents several disadvantages: (1) the radioisotopes ⁵⁹Fe and ⁵⁵Fe decay *via* X- and/or β-ray emission with physical half-lives of 45 days and 2.7 years, respectively; (2) absorbed iron is not actively excreted from and is salvaged in the human body during hematopoiesis (red blood cell production) which raises ethical concerns exposing human subjects to radioactivity over a long time; (3) formation of unknown impurities due to radiolysis of the drug and therefore radiochemical stability might not be determinable; (4) this is in particular the case for SBR759 which is generally insoluble and has a very complex molecular structure; (5) radiation during the Good Manufacturing Practice (GMP) production of the drug, shipping, administration and analysis requires special precautions, facilities and handling procedures to minimize exposure to radioactivity and cross-contamination; (6) the short physical half-life of 45 days of ⁵⁹Fe does not simply allow prolonged synthesis processes under GMP production or keeping retained samples for analysis afterwards; (7) factors contributing to measurement variability have to be minimized as much as possible, and therefore, it is essential that the drug product is labeled homogeneously with the iron isotope by applying GMP production which is extremely difficult to achieve using radioactive iron.

The disadvantages mentioned above can be circumvented by applying the isotope dilution concept using stable iron isotopes, *e.g.* ⁵⁸Fe or ⁵⁷Fe and determination of isotope ratios by MC-ICP-MS. Many clinical iron uptake studies were conducted in the past using stable iron isotopes and applying the isotope



dilution concept, mainly in nutrition research in adults (Whittaker, 1989)¹⁸ (Barrett, 1992)¹⁹ and children (Janghorbani, 1986)²⁰ (Fomon, 1988)²¹ (Fomon, 1989)²² (Woodhead, 1988)²³ (Walczyk, 1997)²⁴ (Vasquez Garibay, 2001).²⁵

2.1.1 Selection of a stable iron isotope label and the degree of labeling. Due to its very low abundance in nature (~0.3%), the stable iron isotope ⁵⁸Fe was chosen to label SBR759, expecting a high sensitivity and a low detection limit of the isotopic label. With the development of highly sensitive MC-ICP-MS instruments (Halliday, 1998),²⁶ (Jakubowski, 2011)²⁷ a low isotope enrichment can be applied. We estimated that additional enrichment of SBR759 with approx. 2.5% ⁵⁸Fe would be sufficient to detect changes in the ⁵⁸Fe/⁵⁶Fe ratio by MC-ICP-MS with a good signal-to-noise ratio and high precision and accuracy in blood and plasma. Low isotope enrichment reduces: (i) the costs for the total amount of iron isotope needed to produce the drug product, and (ii) the potential of introducing associated heavy metal impurities which may lead to possible toxicity.

2.2 Study design

The clinical part of this open-label study was performed at Covance Clinical Research Unit AG (former Swiss Pharma Contract Ltd), Allschwil, Switzerland. This study was approved by the Cantonal Ethics Committee and was conducted in accordance with the declaration of Helsinki (1964 and subsequent revisions) and International Conference on Harmonization-Good Clinical Practice guidelines. The analytical investigations of the ferrokinetics and iron uptake of [⁵⁸Fe]SBR759-related iron were not required to be conducted under GLP but were carried out according to the available current scientific standards.

2.2.1 Subjects. Twelve (12) healthy male volunteers were enrolled and completed the study (Table 1). All subjects gave written informed consent before participating in the study. Each subject participated in a 21 day screening period (Day-21 to Day-2),

a baseline period (Day-1), and a 12 hour dosing period (Day 1, three administrations of 4 g of [⁵⁸Fe]SBR759 each) followed by a 3 week ambulatory period and a study completion on Day 21.

Healthy, nonsmoking men between 18 and 45 years of age were enrolled. Subjects with (i) hematocrit <41%, (ii) hemoglobin <13.8 or >17.2 g dL⁻¹, (iii) serum ferritin <20 or >320 ng mL⁻¹, (iv) transferrin saturation <20%, (v) reticulocyte count >1.5% or platelets <100 000 μL⁻¹, (vi) history of anemia, hemochromatosis or other dyscrasia(s), e.g. thalassemia, myelodysplastic syndrome, etc., treated or not had to be excluded from the study. A total of 12, iron replete i.e., nonanemic, subjects were recruited. This population was selected as their propensity for iron absorption was expected to be similar to that of a CKD patient on dialysis based on similarities in absorption of therapeutic iron salts (Skikne, 2000).²⁸

2.2.2 Study drug

Stable ⁵⁸Fe isotope labeling of SBR759. Research grade ⁵⁸Fe-enriched (99.49%) iron was purchased from Isoflex, San Francisco, CA, USA. ⁵⁸Fe was completely dissolved in 37% (w/w) aqueous hydrochloric acid at ambient temperature. The resulting aqueous FeCl₂-solution was filtered and subsequently oxidized by addition of excess of gaseous chlorine. The resulting aqueous ⁵⁸FeCl₃ solution was evaporated to dryness yielding ⁵⁸FeCl₃·6H₂O as colorless hygroscopic powder (Tessenderlo AG, Bad Zurzach, Switzerland). ⁵⁸FeCl₃·6H₂O was diluted with commercially available FeCl₃·6H₂O (Merck, Darmstadt, Germany) to a target ⁵⁸Fe content of 2.5%. ⁵⁸Fe stable isotope-labeled SBR759 was prepared starting from ⁵⁸Fe-stable isotope-labeled FeCl₃·6H₂O using the same synthesis protocol as for the GMP production of the SBR759 drug substance for human use (Novartis Pharma, AG; Basel, Switzerland).

The drug product (SBR759 ⁵⁸Fe Moda Saccharose, iron aqua carbonate hydroxyl-oxo starch sucrose complex (CAS registration No: 1041180-02-2)) contained 20.4% w/w of iron consisting of 5.706% ⁵⁴Fe, 89.552% ⁵⁶Fe, 2.077% ⁵⁷Fe and 2.665% ⁵⁸Fe isotopes as determined by MC-ICP-MS.

Table 1 Demographic data, administered [⁵⁸Fe]SBR759 dose and the total amount of iron in whole blood of each subject at pre-dose (baseline)

Subject number	Total [⁵⁸ Fe]SBR759 dose (g)	Body weight (kg)	Body height (cm)	Total Fe concentration in whole blood ^{a,b}		Calculated blood volume ^c (mL)	Total amount of Fe in whole blood (mg)
				(ppm)	(μg mL ⁻¹)		
5101	12.47	71.7	174	460	483	4400	2127
5102	12.48	97.8	177	490	515	5320	2735
5103	12.47	86.8	172	450	473	4820	2282
5104	12.49	84.9	179	470	494	4960	2450
5105	12.45	57.2	165	500	525	3690	1937
5106	12.48	78.0	186	460	483	4950	2389
5107	12.42	79.5	182	460	483	4880	2357
5108	12.47	59.2	166	460	483	3780	1827
5109	12.47	67.2	179	450	473	4410	2084
5110	12.46	102.1	186	490	515	5710	2939
5111	12.47	97.4	183	510	536	5470	2934
5112	12.46	81.1	180	510	536	4870	2612
Mean	12.47	80.2	177	475.8	499.9	4770	2389
SD	0.017	14.7	7.0	22.7	24.0	620	365
CV (%)	0.13	18.3	3.9	4.8	4.8	13.0	15.3
Range	12.42–12.49	57.2–102.1	165–186	450–510	473–536	3690–5710	1827–2939

^a As determined by ICP-OES in blood collected at 0 h (pre-dose, baseline). ^b Using a blood density of 1.050 g mL⁻¹ at 37 °C (Geigy Scientific Tables).³⁴ ^c Blood volume calculated according to eqn (12).



Table 2 Molar iron isotope ratios of the [⁵⁸Fe]SBR759 drug product determined by MC-ICP-MS. The iron isotope ratios ⁵⁸Fe/⁵⁶Fe, ⁵⁸Fe/⁵⁴Fe, ⁵⁷Fe/⁵⁴Fe and ⁵⁶Fe/⁵⁴Fe were determined by MC-ICP-MS following eqn (1) relative to the "IRMM-014" iron standard²⁹

	Iron isotope ratios			
	⁵⁸ Fe/ ⁵⁶ Fe	⁵⁸ Fe/ ⁵⁴ Fe	⁵⁷ Fe/ ⁵⁴ Fe	⁵⁶ Fe/ ⁵⁴ Fe
Iron isotope ratio	0.029761	0.46705	0.363943	15.69313
Relative error (2σ; n = 3)	0.000034	0.00054	0.000012	0.00030
CV (%)	0.11	0.12	0.32	0.0019

The molar iron isotope ratios ⁵⁸Fe/⁵⁶Fe, ⁵⁸Fe/⁵⁴Fe, ⁵⁷Fe/⁵⁴Fe and ⁵⁶Fe/⁵⁴Fe measured by MC-ICP-MS in the [⁵⁸Fe]SBR759 drug product are listed in Table 2 as relative to the "IRMM-014" iron reference standard (IRMM-014 Fe Standard).²⁹ The raw data are reported in Online Supplement S4 (von Blanckenburg, submitted).¹⁷

2.2.3 Drug administration. The study drug was provided as open-label medication packaged in sachets each containing a single dose of 4 grams of [⁵⁸Fe]SBR759. Subjects received a total daily dose of 12 g (mean ± SD, n = 12: 12.47 ± 0.017 g; Table 1) which was given in three portions of approx. 4 g (range: 4.12–4.17 g), each. Each portion of the study medication was suspended in water and administered by the study center personnel immediately after breakfast (0 h) after an overnight fast of at least 10 hours, thereafter at lunch (4 h) and dinner (10 h) on Day 1 of dosing. In order to better replicate the expected clinical conditions in patients with CKD patients who are generally prescribed a low phosphorus diet, the subjects in this clinical trial received standardized low phosphorus meals (≤1000 mg phosphate) that excluded: red meat, beans, spinach, citrus fruits or juices during the dosing day. The subjects kept their usual diet habits when not domiciled.

2.2.4 Sample collection. Serial blood samples were collected at 0 h (pre-dose; baseline), 6, 12, 16, 22 (Day 1), 26, 34 (Day 2), 106 (Day 5), 250 (Day 11), 322 (Day 14), and 490 h (Day 21) post first dose.

In order to minimize hemolysis during Day 1 and Day 2 when several blood withdrawals took place, blood samples were collected *via* a plastic cannula placed in a forearm vein that remained through sampling 26 hours post last dose. For blood

sampling at Days 5, 11, 14 and 21 a metallic needle (venipuncture) was used for individual samplings. Blood was collected (0–16 h: 35 mL; 22–490 h: 5 mL) *via* gentle aspiration into special 7.5 mL S-Monovettes[®] for metal analysis containing only a small and specified amount of metal impurities (<50 ng iron per tube; Sarstedt, Germany). These tubes contained about 4–7 μL of a lithium heparin solution corresponding to about 0.1% of the final blood sample volume. After blood collection the tubes were inverted gently several times. Immediately after collection, five aliquots of whole blood were exactly weighed into pre-labeled polypropylene cryotubes and stored at ≤−20 °C.

Plasma was obtained from heparinized blood (0, 6, 12, 16, 22 and 26 h post first dose) by centrifugation at 2000 × g, at 4 °C for 10 min. Plasma was recovered into pre-weighed tubes (S-Monovettes[®] for metal analysis, Sarstedt) and stored at ≤−20 °C. Plasma samples which displayed signs of hemolysis were excluded from data analysis.

2.2.5 Sample storage and shipment conditions. After sample collection, blood and plasma samples were frozen and stored at ≤−20 °C until and after analysis in the analytical laboratories. Samples were shipped to analytical laboratories under dry ice to keep them frozen.

2.2.6 Safety of subjects and drug tolerability assessments. Safety of subjects and drug tolerability assessments involved monitoring and recording of all adverse events (AEs) and serious adverse events (SAEs), with their severity and relationship to the study drug; regular monitoring of hematology, blood chemistry; urinalysis; ECG; regular measurement of vital signs; and the performance of physical examinations. The subjects' iron status was monitored by measuring hemoglobin, hematocrit, ferritin and transferrin saturation (Table 3).

2.3 Iron isotope determination in blood and plasma

2.3.1 MC-ICP-MS. For stable iron isotope analysis by multi-collector ICP-MS and the sample preparation required, we used the protocol described by (Schoenberg, 2005).³⁰ Detailed modifications of that method and the new developments to allow for measurements of stable iron isotopes in blood and plasma are reported in a companion publication to this study (von Blanckenburg, submitted).¹⁷ Briefly, iron extraction from blood and plasma was achieved by microwave irradiation

Table 3 Iron toxicity-specific parameters hemoglobin, hematocrit, ferritin and transferrin saturation (TSAT) measured at screening, baseline and at Days 11 and 21 in blood of 12 healthy male subjects following treatment with an oral dose of 12 g (3 × 4 g) of [⁵⁸Fe]SBR759

Mean ± SD (range)	Iron toxicity-specific laboratory parameters							
	Hemoglobin ^a (mmol L ⁻¹)		Hematocrit (%)		Ferritin (μg L ⁻¹)		TSAT (%)	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Screening (Day-21 to Day-2)	9.7 ± 0.52	9.0–10.6	45.1 ± 2.0	42.5–49.8	94.6 ± 52.9	30–217	34.6 ± 11.2	20.8–54.6
Baseline (Day-1)	9.4 ± 0.48	8.7–10.2	44.0 ± 2.3	40.6–48.7	86.4 ± 56.4	28–219	35.8 ± 19.0	22.0–93.6 ^b
Day 11	8.9 ± 0.65	8.2–9.8	41.9 ± 2.4	38.4–46.2	70.5 ± 43.6	28–167	24.3 ± 8.9	10.1–36.9
Day 21	8.8 ± 0.62	8.0–10.1	41.8 ± 2.8	37.0–47.9	68.3 ± 54.3	30–191	24.0 ± 12.5	10.5–45.2
Normal ranges	8.7	10.9	40.1	51	30	400	16	45

^a To obtain g dL⁻¹ divide nmol L⁻¹ values by 0.6206. ^b Outlier value: not repeated nor excluded from average and standard deviation.



treatment with subsequent ion exchange chromatography. Isotope ratios were determined on a Thermo Finnigan “Neptune” Multicollector ICP-MS at the Institute for Mineralogy from the Leibniz University of Hannover, Germany.

Iron standard for controlling MC-ICP-MS measurements. Commercially available “IRMM-014” iron reference material (from EU Institute for Reference Materials and Measurements, Geel, Belgium) was used as the “bracketing standard” in measurements (Schoenberg, 2005),³⁰ and to calculate normalized isotope ratio differences according to (eqn (1)). Iron in the “IRMM-014” standard is reported to be at a molar weight of $55.84515 \pm 0.00048 \text{ g mol}^{-1}$ consisting of the following isotope mass fractions: $^{54}\text{Fe} = 5.845\%$, $^{56}\text{Fe} = 91.754\%$, $^{57}\text{Fe} = 2.1192\%$ and $^{58}\text{Fe} = 0.2818\%$. For MC-ICP-MS measurements, the “IRMM-014” metallic iron standard was dissolved in 0.3 mol L^{-1} HNO_3 (Merck KGaA, Darmstadt, Germany). Before use, HNO_3 was distilled until content in iron was $<0.2 \text{ ppb}$ (controlled by ICP-OES).

Interference correction of ^{54}Cr on ^{54}Fe and ^{58}Ni on ^{58}Fe . To avoid artifacts introduced by a high Cr or Ni corrections, Fe isotope analyses with $^{54}\text{Cr}/^{54}\text{Fe} > 0.1\%$ were rejected, while $\delta^{58/56}\text{Fe}$ values of samples with $^{58}\text{Ni}/^{58}\text{Fe} > 10\%$ were rejected from the data sets (von Blanckenburg, submitted).¹⁷

2.3.2 ICP-OES. The total iron concentration in blood and plasma was determined by validated inductively coupled plasma optical emission spectroscopy (ICP-OES) on a Varian Vista Pro system (Varian GmbH, Germany). The measurements were carried out using a GMP method (Solvias AG, Basel, Switzerland).

2.4 Data evaluation and equations used

Isotope ratio measurements by ICP-MS never yield absolute ratios. They are affected by a variable instrumental mass bias, the degree of which depends on run conditions. To make the measurements of unknown sample compositions comparable, a reference material of known composition is measured (meas) before and after each sample. In this so-called “bracketing technique” sample ratios are normalized to the nominal (nom) value of the reference material. We measured the “IRMM-014” iron reference standard before and after each sample. The normalized $^{58}\text{Fe}/^{56}\text{Fe}$ ratio of the sample $^{58}\text{Fe}/^{56}\text{Fe}_{\text{sample}}$ then is:

$$\begin{aligned} ^{58}\text{Fe}/^{56}\text{Fe}_{\text{sample}} &= ^{58}\text{Fe}/^{56}\text{Fe}_{\text{IRMM-014,nom}} \\ &\times \frac{^{58}\text{Fe}/^{56}\text{Fe}_{\text{sample,meas}}}{^{58}\text{Fe}/^{56}\text{Fe}_{\text{IRMM-014,meas}}} \end{aligned} \quad (1)$$

As natural variations in the stable isotope composition of an element are usually small, a more convenient reporting of isotope ratios is conventional in Isotope Geochemistry. This so-called delta (δ) notation also accounts for differences in instrumental mass bias, and is used for reporting relative differences in iron isotope ratios between the sample and the reference material. Accordingly, measured $^{58}\text{Fe}/^{56}\text{Fe}$ ratios are reported as $\delta^{58/56}\text{Fe}$ values and usually multiplied by 1000 to

be reported in permil [‰] or parts per thousand ($1\text{‰} = 0.1\%$) according to eqn (1).

$$\frac{\delta^{58/56}\text{Fe}_{\text{sample}}}{\text{‰}} = \left[\frac{^{58}\text{Fe}/^{56}\text{Fe}_{\text{sample}}}{^{58}\text{Fe}/^{56}\text{Fe}_{\text{IRMM-014}}} - 1 \right] \cdot 1000 \quad (2)$$

We use eqn (1) throughout this paper, but for consistency we also report the $\delta^{58/56}\text{Fe}$ values from eqn (2) in the Online Supplement S4 (von Blanckenburg, submitted).¹⁷ By using $^{58}\text{Fe}/^{54}\text{Fe}$ isotope ratios for calculation, similar ferrokinetics and absorption results are obtained.

The molar amount of absorbed [^{58}Fe]SBR759-related iron n_{iso} is calculated by eqn (3), derived from (Walczyk, 1997).²⁴

$$n_{\text{iso}} = n_{\text{nat}} \cdot \frac{a_{\text{nat}}^{58} - R_{58/56,\text{sample}} \cdot a_{\text{nat}}^{58}}{R_{58/56,\text{sample}} \cdot a_{\text{iso}}^{56} - a_{\text{iso}}^{58}} \quad (3)$$

n_{nat} , the molar amount of iron of natural composition (calculated with Fe concentration (Fe_{conc}) in blood and the atomic weight of natural iron ($\text{awt}_{\text{Fe,nat}}$) at baseline ($t = 0 \text{ h}$); a_{nat}^{58} , the natural isotopic abundance of ^{58}Fe at baseline; a_{nat}^{56} , the natural isotopic abundance of ^{56}Fe at baseline; a_{iso}^{58} , the isotopic abundance of ^{58}Fe in SBR759; a_{iso}^{56} , the isotopic abundance of ^{56}Fe in SBR759; $R_{58/56,\text{sample}}$, the $^{58}\text{Fe}/^{56}\text{Fe}$ ratio relative to “IRMM-014” iron standard, calculated using eqn (1).

The atomic weight of iron of natural composition ($\text{awt}_{\text{Fe,nat}}$) is calculated for each subject by eqn (4):

$$\text{awt}_{\text{Fe,nat}} = m_{54} \cdot a_{\text{nat}}^{54} + m_{56} \cdot a_{\text{nat}}^{56} + m_{57} \cdot a_{\text{nat}}^{57} + m_{58} \cdot a_{\text{nat}}^{58} \quad (4)$$

m_{5x} , the atomic weight of ^{5x}Fe (DeLaeter, 2003);³¹ a_{nat}^{5x} , the natural abundance of ^{5x}Fe ($x = 4, 6, 7$ and 8) at baseline; The latter is calculated by eqn (5) and (6):

$$a_{\text{nat}}^{56} = \frac{1}{1 + R_{54/56,\text{nat}} + R_{57/56,\text{nat}} + R_{58/56,\text{nat}}} \quad (5)$$

$$a_{\text{nat}}^{5x} = a_{\text{nat}}^{56} \cdot R_{5x/56,\text{nat}} \quad (6)$$

$R_{5x/56,\text{nat}}$, the $^{5x}\text{Fe}/^{56}\text{Fe}$ ratio relative to the “IRMM-014” iron standard according to eqn (2), measured at baseline ($x = 4, 7$ and 8).

The abundances of the iron isotopes in SBR759 (a_{iso}^{5x}) are calculated by eqn (7) and (8):

$$a_{\text{iso}}^{56} = \frac{1}{1 + R_{54/56,\text{iso}} + R_{57/56,\text{iso}} + R_{58/56,\text{iso}}} \quad (7)$$

$$a_{\text{iso}}^{5x} = a_{\text{iso}}^{56} \cdot R_{5x/56,\text{iso}} \quad (8)$$

$R_{5x/56,\text{iso}}$, the $^{5x}\text{Fe}/^{56}\text{Fe}$ ratio relative to the “IRMM-014” iron standard according to eqn (2) of SBR759 ($x = 4, 7$ and 8).

The molar amount of absorbed [^{58}Fe]SBR759-related iron (n_{iso}) can be converted to the amount of adsorbed iron in mg (m_{iso}) by eqn (9):

$$m_{\text{iso}} = n_{\text{iso}} \cdot \text{awt}_{\text{Fe,iso}} \cdot 1000 \quad (9)$$

The atomic weight of iron in SBR759 ($\text{awt}_{\text{Fe,iso}}$) is calculated by eqn (10):

$$\text{awt}_{\text{Fe,iso}} = m_{54} \cdot a_{\text{iso}}^{54} + m_{56} \cdot a_{\text{iso}}^{56} + m_{57} \cdot a_{\text{iso}}^{57} + m_{58} \cdot a_{\text{iso}}^{58} \quad (10)$$



Due to the long life span of erythrocytes (~ 120 days), the total amount of absorbed [^{58}Fe]SBR759-related iron can be determined 14 days (or if more conservative 21 days) after the administration. Since not all of the absorbed [^{58}Fe]SBR759-related iron is incorporated into erythrocytes, an incorporation factor (f_{inc}) is included (Walczyk, 1997).²⁴ According to (Bernat, 1983),³² 70–90% of absorbed [^{58}Fe]SBR759-related iron is incorporated into erythrocytes. Therefore, f_{inc} will be set to the mean value of 80%. The final, absolute amount of absorbed [^{58}Fe]SBR759-related iron m_{abs} is calculated by eqn (11):

$$m_{\text{abs}} = \frac{m_{\text{iso}}}{f_{\text{inc}}} \quad (11)$$

We note that during intestinal absorption light isotopes are preferred by mass-dependent isotope fractionation. For European male omnivores this fractionation amounts to a shift in the $^{58}\text{Fe}/^{56}\text{Fe}$ ratio by a factor of 0.9985 (von Blanckenburg, 2013).³³ As this shift is negligible when compared to the shifts in $^{58}\text{Fe}/^{56}\text{Fe}$ introduced by [^{58}Fe]SBR759, we ignore this absorption effect.

The blood volume (BV; in mL) is calculated by eqn (12) (Geigy Scientific Tables, 1984)³⁴ which is based on the body length (L ; in cm) and the body mass (B ; in kg) (Table 1):

$$\text{BV} = L \times 28.5 + B \times 31.6 - 2820 \quad (12)$$

The corresponding plasma volume is calculated by multiplying the calculated blood volume BV with the blood/plasma volume ratio of 0.5769 calculated from Table II in (Davies, 1993).³⁵

3 Results and discussion

3.1 Safety of subjects and drug tolerability assessment

Doses of 12 g ^{58}Fe -labeled SBR759, divided in three doses of 4 g, each, and administered with a low phosphorus meal, were generally well tolerated by healthy male subjects. There was no serious adverse event reported in the study. The most common adverse events were flatulence, discolored feces, diarrhea, and headache which were mild or moderate and transient. There were no clinically significant changes in laboratory parameters, vital signs, ECGs, or physical examinations over the course of the study. There was no sign of overt or acute iron toxicity as there were no clinically significant changes in laboratory parameters hemoglobin, hematocrit, ferritin and transferrin saturation (TSAT) (Table 3).

The mean TSAT values at Days-1, 11 and 21 were 35.8, 24.3 and 24.0% which were in the normal range (16–45%) and distinctly below the toxic limit of 85% leading to labile redox active plasma iron (LPI). It should be noted that an obvious TSAT outlier of 93.6% at baseline (prior to treatment) was reported for subject 5110. This value was not repeated nor excluded from the listing. There was no impact on the mean *versus* median values, therefore mean values are reported.

There was a trend to numerically lower mean and median hemoglobin, hematocrit, ferritin and TSAT values. These trends were likely due to the volume of blood drawn from subjects in

the immediate post dose time period (~ 246 mL) which amounts to ~ 108 mg of iron lost during the first 26 hours of the study and ~ 132 mg iron during the entire study ($300 \text{ mL} \times \text{Hct} \times 1 \text{ mg mL}^{-1}$). These losses occurred in the time period after dosing. Hence they are not expected to drive iron absorption as it is expected that the ^{58}Fe -isotope-labeled-SBR759 would have passed through the proximal small bowel before the blood/iron losses would have stimulated the need to iron absorption. Therefore this is not expected to have an impact on the conclusion of iron absorption from SBR759.

These safety results in healthy volunteers are in agreement with those of three clinical trials in chronic kidney disease patients on hemodialysis (Block, 2010)⁹ (Chen, 2011)¹⁰ (Fukagawa, 2014)¹¹ where SBR759 was well tolerated within the anticipated clinical dose range of 3.75 to 15 g SBR759 per day. No treatment-related serious adverse events were observed nor were there clinically relevant changes in iron indices.

3.2 Ferrokinetics in blood and plasma

The ferrokinetics of SBR759-related iron in whole body blood of the individual subjects was investigated in the time period from 1 to 21 days (0–490 h) post [^{58}Fe]SBR759 dose (Fig. 1).

In order to monitor the rapid systemic uptake of [^{58}Fe]SBR759-related iron into the bloodstream, also whole body blood and plasma in the early time window between 0 and 26 hours were investigated (Fig. 2A and B).

The calculation of total blood iron and plasma iron was based on $^{58}\text{Fe}/^{56}\text{Fe}$ isotope ratios. In contrast to blood, plasma $^{58}\text{Fe}/^{56}\text{Fe}$ and $^{58}\text{Fe}/^{54}\text{Fe}$ ratio values were very high due to the 320- to 590-fold lower amount of iron in plasma (adult males: 0.75–1.75 mg L^{-1}) (Geigy Scientific Tables, 1984).³⁴ Within 0–26 h, most subjects reached a first maximum at 12 hours amounting to 0.22 to 2.3 mg

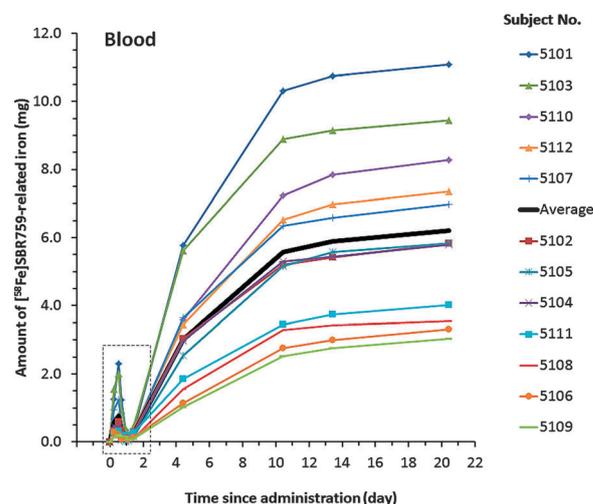


Fig. 1 Amount of total [^{58}Fe]SBR759-related iron in whole body blood collected between Day 1 and Day 21 post 12 g (3×4 g) of [^{58}Fe]SBR759 dose to 12 healthy male subjects. The amount of total blood iron was determined by MC-ICP-MS. Total blood iron calculation based on $^{58}\text{Fe}/^{56}\text{Fe}$ isotope ratios. The time window between 0 and 26 hours (dashed rectangle) is shown enlarged in Fig. 2A. Almost identical total blood iron values were calculated by using $^{58}\text{Fe}/^{54}\text{Fe}$ isotope ratios (not shown).



in blood (Fig. 2A) and 0.15 to 1.8 mg in plasma (Fig. 2B). Thereafter, total blood and plasma iron values dropped by about 26 hours to low levels of 0.01–0.22 mg in blood and 0.02 to 0.18 mg in plasma. Comparable to our study, healthy volunteers treated with oral phosphate binders combined with supplemental iron salts reached plasma iron plateaus by about 6 hours following dosing (Pruchnicki, 2002).³⁶ In our study the ferrokinetic curves were formed out of three single curves related to each of the three consecutive [⁵⁸Fe]SBR759 doses given at 0, 4 and 10 hours. The early maximum at approx. 12 hours can be attributed to a transient appearance of transferrin-bound SBR759-related iron in blood and plasma. After 26 hours, SBR759-related total blood iron steadily increased, each subject reaching a different plateau between 14 and 21 days (Fig. 1). Total SBR759-related blood iron ranged between 3.03 and 11.1 mg revealing high inter-subject variability (CV: 41–104%).

Apart from the upstream intestinal enterocytes, blood plasma is the primary central compartment for iron absorption since virtually all cells in the organism take up iron from transferrin (the major iron transporter protein: apo-transferrin containing bound iron) (Walczyk, 2005).¹⁶ Iron transport-capacity of transferrin is limited and transferrin-bound iron turnover is relatively

short-lived due to the quick transfer of freshly absorbed iron to other tissue compartments, *i.e.*, mainly to the bone marrow for erythrocyte synthesis but also to liver and muscle tissues.

3.3 Extent of systemic iron absorption

The absorption of [⁵⁸Fe]SBR759-related iron was investigated in blood samples collected at baseline and compared to those measured 14 and 21 days after administration (Table 4) (Fig. 1).

Total blood iron was measured by MC-ICP-MS and applying the stable isotope dilution principle. Assuming that approx. 80% of absorbed SBR759-related iron is incorporated into erythrocytes (Bernat, 1983),³² the amount of absorbed iron ranged from 3.8 mg (subject 5109) to 13.9 mg (subject 5101) at Day 21 following [⁵⁸Fe]SBR759 administration. The arithmetic mean was 7.8 mg which corresponds to about 0.3% of the iron dose in SBR759. Compared to the daily iron uptake of 1 to 2 mg in healthy male adults, a mean uptake of 7.8 mg appears to be relatively high. An extrapolation of these data to the target CKD patient population is tainted with uncertainties because in contrast to healthy subjects, CKD patients do not absorb orally administered iron salts well (Hörl, 2007).³⁷

In a recent iron absorption study (Geisser, 2010)³⁸ with 10 g ⁵⁹Fe-radiolabeled PA21 (an iron-based polynuclear iron(III) oxy-hydroxide compound), which was administered to CKD patients and healthy volunteers (HVs), the HVs have taken up 8.3 mg iron, and CKD patients 10-fold less (~0.8 mg). This malabsorption has been linked to the inflammatory state associated with CKD and up-regulation of hepcidin expression

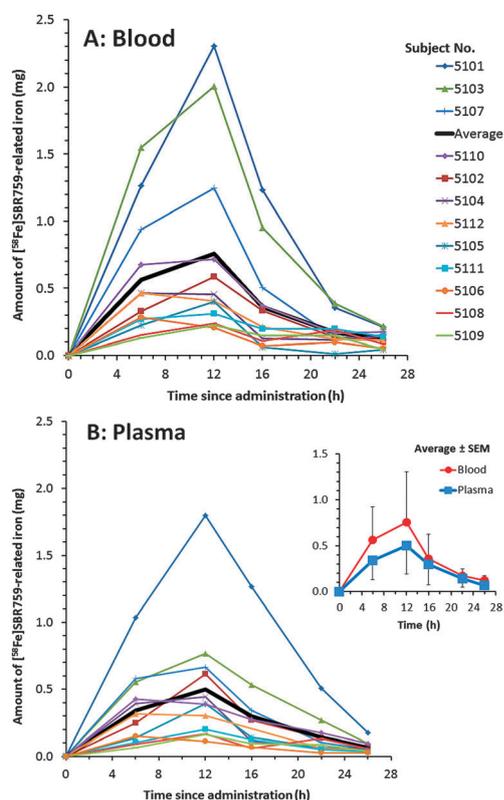


Fig. 2 Amount of total [⁵⁸Fe]SBR759-related iron in whole blood (A) and plasma (B) during the early time period 0–26 hours post 3 × 4 g [⁵⁸Fe]SBR759 dose to 12 healthy male subjects. The amount of iron in blood and plasma in individual subjects (inset: average ± SEM in blood and plasma over *n* = 12 subjects) was determined by MC-ICP-MS. Amount of iron calculation based on ⁵⁸Fe/⁵⁶Fe isotope ratios. Almost identical total blood and plasma iron values were calculated by using ⁵⁸Fe/⁵⁴Fe isotope ratios (not shown).

Table 4 Calculated amounts of systemically absorbed iron in whole blood of healthy male subjects measured at Days 14 and 21 following oral dosing of 12 g (3 × 4 g) of [⁵⁸Fe]SBR759

Subject	Blood collected after 14 days		Blood collected after 21 days	
	Calculated amount of absorbed iron	Relative to total iron dose (%)	Calculated amount of absorbed iron	Relative to total iron dose (%)
5101	13.4	0.53	13.9	0.54
5102	6.8	0.27	7.3	0.29
5103	11.5	0.45	11.8	0.46
5104	6.8	0.27	7.3	0.28
5105	7.0	0.27	7.3	0.28
5106	3.8	0.15	4.1	0.16
5107	8.2	0.33	8.7	0.34
5108	4.3	0.17	4.4	0.17
5109	3.5	0.14	3.8	0.15
5110	9.8	0.39	10.4	0.41
5111	4.7	0.18	5.0	0.20
5112	8.7	0.34	9.2	0.36
Arithmetic mean	7.4	0.29	7.8	0.30
Median	6.9	0.27	7.3	0.29
Minimum	3.5	0.14	3.8	0.15
Maximum	13.4	0.53	13.9	0.54
First quartile (25%)	4.6	0.18	4.9	0.19
Third quartile (75%)	9.0	0.35	9.5	0.37
Standard deviation	3.1	0.12	3.2	0.12
CV (%)	42	41	41	40



(Hörl, 2007)³⁷ (Andrews, 2007).³⁹ Although there is a hypothetical risk for local toxicity of SBR759 with chronic administration, the assessment of such a risk is out of scope for this abbreviated trial and is a more appropriate question for chronic toxicology studies in animals and additional clinical testing in the target patient population which would be required prior to health authority approval (Block, 2010)⁹ (Chen, 2011)¹⁰ (Fukagawa, 2014).¹¹ In addition, considering the low bioavailability of iron from the SBR759 drug substance compared to that expected from therapeutic oral iron supplements (*e.g.* ferrous sulfate) which are administered by thousands of patients per day, the risk of ROS-related GIT damage appears to be minimal.

4 Conclusions

- The results of this phase I mechanistic iron uptake study provide insight into the potential for iron absorption from a daily dose of 12 g (divided in 3 × 4 g) of [⁵⁸Fe]SBR759 to healthy male subjects.
 - The study demonstrated absorption of 0.15 to 0.54% of the total daily administration of [⁵⁸Fe]SBR759-related iron which amounted on average to 7.8 mg; approx. 5-fold more than the basal daily iron absorption (1 to 2 mg).
 - [⁵⁸Fe]SBR759-related iron transiently appeared in blood and plasma within the first 26 hours post first dose, followed by second later phase in blood ranging over 3 weeks which is characterized by redistribution of [⁵⁸Fe]SBR759-related iron from blood into other relevant iron storage tissue compartments such as bone marrow, liver and muscle.
 - In an interdisciplinary collaboration of the pharmaceutical industry with the academic environment in the field of Geosciences, the MC-ICP-MS technique was successfully applied for reliably measuring iron absorption and ferrokinetics in human blood and plasma with high specificity, sensitivity, accuracy and precision.

Abbreviations

CKD	Chronic kidney disease
GMP	Good manufacturing practice
ICP-OES	Inductively coupled plasma optical emission spectroscopy
MC-ICP-MS	Multi-collector inductively coupled plasma mass spectrometry.

Authors contribution

Authors in alphabetical order.

Participated in research design: Gschwind, Schmid, Slade, Stitah, Swart, von Blanckenburg. *Conducted experiments:* Kaufmann, van Zuilen, Oelze, Schmid, Stitah. *Performed data analysis:* Gschwind, van Zuilen, Oelze, Schmid, von Blanckenburg. *Wrote or contributed to the writing of the manuscript:* Gschwind, Kaufmann, Schmid, Slade, Stitah, Swart, von Blanckenburg.

Acknowledgements

Michael Seiberling, MD, Clinical Investigator (Covance Clinical Research Unit AG, Switzerland). Andreas Helfrich, PhD, (Solvias AG, Analytics, Basel, Switzerland) for ICP-OES measurements. Zdenek Zencak, PhD, (Technical Research and Development, Novartis, Basel, Switzerland) for analyzing the [⁵⁸Fe]SBR759 drug product. Prof. Kaspar Hegetschweiler, PhD, (Universität des Saarlandes, Germany) for scientific consultancy, Prof. Jochen Litterst, PhD, (Technische Universität Braunschweig, Germany) for Mössbauer spectroscopic investigations and Ernst Küsters, PhD, (Novartis Basel, Switzerland) for investigations of the redox activity of SBR759. Hendrik Andres, PhD, and Thomas Moenius, PhD, for scientific advices and managing the purchase order of ⁵⁸Fe metal (Isotope Laboratory, Novartis Basel, Switzerland). We acknowledge Ingo Horn, PhD, (Leibniz Universität Hannover, Germany) for supporting mass spectrometric measurements, Prof. Ronny Schoenberg, PhD, (University Tübingen, Germany) for advice in the design of this study. We thank Prof. Thomas Walczyk, PhD, (National University of Singapore) and an anonymous manuscript reviewer for their constructive comments.

References

- 1 A. J. Bleyer, S. K. Burke, M. Dillon, B. Garrett, K. S. Kant, D. Lynch, S. N. Rahman, P. Schoenfeld, I. Teitelbaum, S. Zeig and E. Slatopolsky, A comparison of a calcium free phosphate binder sevelamer hydrochloride with calcium acetate in the treatment of hyperphosphatemia in haemodialysis patients, *Am. J. Kidney Dis.*, 1999, **33**, 694–701.
- 2 P. C. D'Haese, G. B. Spasovski, A. Sikole, A. Hutchison, T. J. Freemont, S. Sulkova, C. Swanepoel, S. Pejanovic, L. Djukanovic, A. Balducci, G. Coen, W. Sulowicz, A. Ferreira, A. Torres, S. Curic, M. Popovic, N. Dimkovic and M. E. De Broe, A multicenter study on the effects of lanthanum carbonate (Fosrenol™) and calcium carbonate on renal bone disease in dialysis patients, *Kidney Int.*, 2003, **63**, S73–S78.
- 3 B. Brezina, W. Y. Qunibi and C. R. Nolan, Acid loading during treatment with sevelamer hydrochloride: mechanisms and clinical implications, *Kidney Int.*, 2004, **66**(suppl 90), S39–S45.
- 4 A. J. Hutchison, Oral phosphate binders, *Kidney Int.*, 2009, **75**(9), 906–914.
- 5 A. J. Hutchison, B. Maes, J. Vanwalleghem, G. Asmus, E. Mohamed, R. Schmieder, W. Backs, R. Jamar and A. Vosskuhler, Efficacy, tolerability, and safety of lanthanum carbonate in hyperphosphatemia: a 6 month, randomized, comparative trial *versus* calcium carbonate, *Nephron*, 2005, **100**, c8–c19.
- 6 E. Slatopolsky, H. Liapis and J. Finch, Progressive accumulation of lanthanum in the liver of normal and uremic rats, *Kidney Int.*, 2005, **68**, 2809–2813.
- 7 G. B. Spasovski, A. Sikole, S. Gelev, J. Masin-Spasovska, T. Freemont, I. Webster, M. Gill, C. Jones, M. E. De Broe and P. C. D'Haese, Evolution of bone and plasma



- concentration of lanthanum in dialysis patients before, during 1 year of treatment with lanthanum carbonate and after 2 years of follow-up, *Nephrol., Dial., Transplant.*, 2006, **621**, 2217–2224.
- 8 (a) O. Hergesell and E. Ritz, Stabilized polynuclear iron hydroxide is an efficient oral phosphate binder in uraemic patients, *Nephrol., Dial., Transplant.*, 1999, **14**, 863–867; (b) O. Hergesell and E. Ritz, Phosphate binders on iron basis: a new perspective?, *Kidney Int.*, 1999, 73(suppl), S42–S45.
 - 9 G. A. Block, S. L. Brillhart, M. S. Persky, A. Amer and A. J. Slade, Efficacy and safety of SBR759, a new iron-based phosphate binder, *Kidney Int.*, 2010, **77**(10), 897–903.
 - 10 J. B. Chen, S. S. Chiang, H. C. Chen, S. Obayashi, M. Nagasawa, J. M. Hexham, A. Balfour, G. Junge, T. Akiba and M. Fukagawa, Efficacy and safety of SBR759, a novel calcium-free, iron(III)-based phosphate binder, in Asian patients undergoing hemodialysis: A 12 week, randomized, open-label, dose-titration study versus sevelamer hydrochloride, *Nephrology*, 2011, **16**(8), 743–750.
 - 11 M. Fukagawa, H. Kasuga, D. Joseph, H. Sawata, G. Junge, A. Moore and T. Akiba, Efficacy and safety of SBR759, a novel calcium-free, iron(III)-based phosphate binder, versus placebo in chronic kidney disease stage V Japanese patients on maintenance renal replacement therapy, *Clin. Exp. Nephrol.*, 2014, **18**(1), 135–143, DOI: 10.1007/s10157-013-0815-7.
 - 12 L. Sigg and W. Stumm, The interaction of anions and weak acids with the hydrous goethite (α -FeOOH) surface, *Colloids Surf.*, 1980, **2**(2), 101–117.
 - 13 R. L. Parfitt, R. J. Atkinson and R. S. C. Smart, The mechanism of phosphate fixation by iron oxides, *Soil Sci. Soc. Am. Proc.*, 1975, **39**, 837–841.
 - 14 G. J. Anderson, Mechanisms of iron loading and toxicity, *Am. J. Hematol.*, 2007, **82**, 1128–1131.
 - 15 V. Chávez, A. Mohri-Shiomi, A. Maadani, L. A. Vega and D. A. Garsin, Oxidative Stress Enzymes Are Required for DAF-16-Mediated Immunity Due to Generation of Reactive Oxygen Species by *Caenorhabditis elegans*, *Genetics*, 2007, **176**(3), 1567–1577.
 - 16 T. Walczyk and F. von Blanckenburg, Deciphering the iron isotope message of the human body, *Int. J. Mass Spectrom.*, 2005, **242**(2–3), 117–134.
 - 17 F. von Blanckenburg, M. Oelze, D. G. Schmid, K. van Zuilen, H.-P. Gschwind, A. J. Slade, S. Stitah, D. Kaufmann and P. Swart, An iron stable isotope comparison between human erythrocytes and plasma, *Metallomics*, DOI: 10.1039/C4MT00124A.
 - 18 P. G. Whittaker, T. Lind, J. G. Williams and A. L. Gray, Inductively coupled plasma mass spectrometric determination of the absorption of iron in normal women, *Analyst*, 1989, **114**(6), 675–678.
 - 19 J. F. R. Barrett, P. G. Whittaker, J. G. Williams and T. Lind, Absorption of non-heme iron in normal women measured by the incorporation of two stable isotopes into erythrocytes, *Clin. Sci.*, 1992, **83**(2), 213–219.
 - 20 M. Janghorbani, B. T. G. Ting and S. J. Fomon, Erythrocyte incorporation of ingested stable isotope of iron (^{58}Fe), *Am. J. Hematol.*, 1986, **21**(3), 277–288.
 - 21 S. J. Fomon, M. Janghorbani, B. T. Ting, E. E. Ziegler, R. R. Rogers, S. E. Nelson, L. S. Ostedgaard and B. B. Edwards, Erythrocyte incorporation of ingested ^{58}Fe iron by infants, *Pediatr. Res.*, 1988, **24**(1), 20–24.
 - 22 S. J. Fomon, E. E. Ziegler, R. R. Rogers, S. E. Nelson, B. B. Edwards, D. G. Guy, J. C. Erve and M. Janghorbani, Iron absorption from infant foods, *Pediatr. Res.*, 1989, **26**(3), 250–254.
 - 23 J. C. Woodhead, J. M. Drulis, R. R. Rogers, E. E. Ziegler, P. J. Stumbo, M. Janghorbani, B. T. Ting and S. J. Fomon, Use of the stable isotope, ^{58}Fe , for determining availability of nonheme iron in meals, *Pediatr. Res.*, 1988, **23**(5), 495–499.
 - 24 T. Walczyk, L. Davidsson, N. Zavaleta and R. F. Hurrell, Stable isotope labels as a tool to determine the iron absorption by Peruvian school children from a breakfast meal, *Fresenius' J. Anal. Chem.*, 1997, **359**(4–5), 445–449.
 - 25 E. Vasquez Garibay, I. Santos Torres, S. E. Nelson, E. E. Ziegler, R. R. Rogers, M. Janghorbani and S. J. Fomon, Iron absorption during recovery from malnutrition, *J. Am. Coll. Nutr.*, 2001, **20**(4), 286–292.
 - 26 A. N. Halliday, D. C. Lee, J. N. Christensen, M. Rehkämper, W. Yi, X. Luo, C. M. Hall, C. J. Ballentine, T. Pettke and C. Stirling, Applications of multiple collector-ICPMS to cosmochemistry, geochemistry and paleoceanography, *Geochim. Cosmochim. Acta*, 1998, **62**, 919–940.
 - 27 N. Jakubowski, T. Prohaska, F. Vanhaecke, P. H. Roos and T. Lindemann, Inductively coupled plasma- and glow discharge plasma-sector field mass spectrometry, *J. Anal. At. Spectrom.*, 2011, **26**, 727–757.
 - 28 B. S. Skikne, N. Ahluwalia, B. Fergusson, A. Chonko and J. D. Cook, Effects of erythropoietin therapy on iron absorption in chronic renal failure, *J. Lab. Clin. Med.*, 2000, **135**(6), 452–458.
 - 29 Institute for Reference Materials and Measurements, European Commission Joint Research Centre, Geel, Belgium, https://irmm.jrc.ec.europa.eu/refmat_pdf/IRMM-014_cert.pdf.
 - 30 R. Schoenberg and F. von Blanckenburg, An assessment of the accuracy of stable Fe isotope ratio measurements on samples with organic and inorganic matrices by high-resolution multicollector ICP-MS, *Int. J. Mass Spectrom.*, 2005, **242**(2–3), 257–272.
 - 31 J. R. DeLaeter, J. K. Boehlke, P. De Bièvre, H. Hidaka, H. S. Peiser, K. J. R. Rosman and P. D. P. Taylor, Atomic weights of the elements: Review 2000 (IUPAC technical report), *Pure Appl. Chem.*, 2003, **75**(6), 683–800.
 - 32 I. Bernat, Incorporation of radioiron into the erythroblasts and reticulocytes. Iron utilization in the course of red cell production. Effective erythropoiesis, in *Iron Metabolism*, ed. A. Kiado, Plenum Press, New York, 1983, ch. 14, pp. 167–181.
 - 33 F. von Blanckenburg, J. Noordmann and M. Guelke-Stelling, The iron stable isotope fingerprint of the human diet, *J. Agric. Food Chem.*, 2013, **61**, 11893–11899.



- 34 Geigy, Scientific Tables, *Geigy Scientific Tables*, ed. C. Lentner, Physical Chemistry, Composition of blood, Hematology, Somatometric Data, Ciba-Geigy Limited, Medical Education Division, Ciba-Geigy Corporation, Basle, Switzerland, West Caldwell, New Jersey, 8th edn, 1984, vol. 3, pp. 65–67 and pp. 84–85.
- 35 B. Davies and T. Morris, Physiological parameters in laboratory animals and humans, *Pharm. Res.*, 1993, **10**(7), 1093–1095.
- 36 M. C. Pruchnicki, J. D. Coyle, S. Hoshaw-Woodard and W. H. Bay, Effect of phosphate binders on supplemental iron absorption in healthy subjects, *J. Clin. Pharmacol.*, 2002, **42**(10), 1171–1176.
- 37 W. H. Hörl, Iron therapy for renal anemia: how much needed, how much harmful, *Pediatr. Nephrol.*, 2007, **22**, 480–489.
- 38 P. Geisser and E. Philipp, PA21: A novel phosphate binder for the treatment of hyperphosphatemia in chronic kidney disease, *Clin. Nephrol.*, 2010, **74**(1), 4–11.
- 39 N. C. Andrews, in *Principles of Molecular Medicine*, ed. M. S. Runge and C. Patterson, Iron metabolism, Humana Press, Inc., Totowa, NJ, 2nd edn, 2007, ch. 87.

