Official Title: **Multicenter, Open-label Active-controlled Randomized Study of Efficacy and Safety of Ferrum Lek® (Iron (III) Hydroxide Polymaltosate), 100 mg Chewable Tablets (Lek d.d., Slovenia) Compared With Maltofer® (Iron (III) Hydroxide Polymaltosate), 100 mg Chewable Tablets (Vifor S.A., Switzerland), in Treatment of Patients With Mild and Moderate Iron-deficiency Anaemia**

NCT Number: NCT03993288

Document Date: Clinical Study Protocol Version 2.0: 20 March 2019

CLINICAL STUDY PROTOCOL

Study title: Multicenter, open-label active-controlled randomized study of efficacy and safety of Ferrum Lek® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia) compared with Maltofer® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Vifor S.A., Switzerland), in treatment of patients with mild and moderate iron-deficiency anaemia.
ID: TE_005_FER_CHT
Protocol version: 2.0 of March 20, 2019
Study drug: Ferrum Lek®
International non-proprietary name: iron (III) hydroxide polymaltosate
Dosage Form: chewable tablets
Study phase: III
Study sponsored by: Sandoz CJSC

Sponsor's Protocol Approval Page

Protocol Title: Multicenter, open-label active-controlled randomized study of efficacy and safety of Ferrum Lek® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia) compared with Maltofer® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Vifor S.A., Switzerland), in treatment of patients with mild and moderate iron-deficiency anaemia.
ID: TE_005_FER_CHT
Protocol version: 2.0 of March 20, 2019
Sponsor's Representative:

[redacted]
(Full name of the authorized person)

[redacted]
(position)

Name of organization:	Sandoz CJSC, Russia
Address:	72 Leningradsky Prospekt, bld. 3, *125317* Moscow, *Russia*
Telephone:	[redacted] (ext. [redacted])
E-mail:	[redacted]

_____ _____ 201__

Signature Date

Investigator's Consent to the Study Protocol

I confirm that I have read and understood this protocol, the Investigator's brochure, including potential risks and side effects of the drug, and other information on the product and study provided by the Sponsor.

I agree to conduct this study in accordance with the requirements of this protocol, and also to protect the rights, safety, confidentiality and well-being of subjects' health in accordance with the ethical requirements set forth in the Declaration of Helsinki of the WMA, in Federal Law N 61-FZ of April 12, 2010 On Circulation of Medicinal Products; requirements of the order of the Ministry of Health of the Russian Federation N 200n of April 01, 2016 On the Approval of the Rules of Good Clinical Practice; the principles of the National Standard of the Russian Federation GOST 52379-2005 Good Clinical Practice (GCP) and other regulatory requirements of the Russian Federation.

I agree to make amendments to the protocol only after notifying the Sponsor, except where necessary to protect the safety, rights, and well-being of subjects. I fully understand that any changes made by the Investigator(s) without preliminary discussion with the Sponsor's representative will constitute a violation of the protocol (except for those procedures that are necessary to preserve the subjects' health).

I agree to personally conduct or supervise the described study.

I agree to inform subjects that the drugs are being used for research purposes; I will ensure compliance with the requirements for obtaining informed consent, after the approval of the Institutional Review Board and the local Independent Ethics Committee (IEC) and in accordance with the principles of GCP.

I agree to report to the Sponsor adverse events that occur in the course of the study in accordance with the principles of GCP.

I agree to ensure that all associates, colleagues, and employees engaged in the study are informed about their obligations to meet the above commitments. I agree to maintain adequate and accurate records, and to provide these records for analysis in accordance with the principles of GCP.

I will ensure that the local IEC, which operates in accordance with the requirements of GCP, is responsible for carrying out the ethical review, as well as for approving the study. I also agree to promptly report to the local IEC all changes in the research activity and all unanticipated problems involving risks to subjects or other issues. Moreover, I will not make any changes to the study without IRB/local IEC, except for necessary cases to address the apparent sudden threats to the life and health of subjects.

I am ready to provide direct access to the primary documents and agree to audit by the auditors from the Sponsor's representatives and the supervisory bodies. I guarantee that the study product(s) supplied by the Sponsor will be used only as described in this protocol.

I agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements of Good Clinical Practice.

Investigator:

Signature: _____

Date: _____ ____, 201__

Full Name (surname, name, patronymic):

Position:

Institution:

Address:

Table of Contents

List of abbreviations

Abbreviation	Explanation
ANOVA	Analysis of variance
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HFE	Hemochromatosis gene
ICH	International Conference on Harmonization
ITT	Intention-to-treat
LD_{100}	Lethal dose, the average dose of the substance in milligrams per kilogram of live weight, which causes the death of 100% of experimental animals
LD_{50}	Half-lethal dose, the average dose of the substance in milligrams per kilogram of live weight, which causes the death of 50% of experimental animals
MLE	Maximum likelihood estimation
PP	Per protocol
BP	Blood pressure
ALT	Alanine aminotransferase
JSC	Joint-stock company
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical Classification System
VAS	Visual analogue scale
HIV	Human immunodeficiency virus
IUS	Intrauterine system
IUD	Intrauterine device
Hb	Hemoglobin
GOST	State standard
GOST R	State standard of the Russian Federation
DBP	Diastolic blood pressure
ID	Iron deficiency
CI	Confidence interval
DMPO	5,5-Dimethyl-1-pyrroline-N-oxide
IDA	Iron deficiency anemia
GI	Gastrointestinal
GIT	Gastrointestinal tract
CRF	Case record form
CRO	Contract research organization
LDL	Low-density lipoprotein
MoH	Ministry of Health
MIC	Minimum inhibitory concentration
ICD	International Classification of Diseases
GCP	Good Clinical Practice
IEC	Independent Ethics Committee
AE	Adverse event
SPC	Summary of Product Characteristics
PVC	Polyvinylchloride
IHPC	Iron(III)-hydroxide polymaltose complex

IPC	Iron polymaltose complex
PND	Postnatal days
PCR	Polymerase chain reaction
RMSAH/RSC	The Russian Medical Society on Arterial Hypertension/the Russian Society of Cardiology
ITP	Iron transport proteins 1 and 2
RE	Reticuloendothelial (cells)
RES	Reticuloendothelial system
SBP	Systolic blood pressure
SAE	Serious adverse event
SOD	Superoxide dismutase
SOP	Standard operating procedure
TF	Transferrin
TfR	Transferrin receptor
FL	Federal law
Full name	First name, patronymic, last name
CNS	Central nervous system
CP	Ceruloplasmin
RR	Respiratory rate
HR	Heart rate
ECG	Electrocardiography

Synopsis

Study title	Multicenter, open-label active-controlled randomized study of efficacy and safety of Ferrum Lek® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia) compared with Maltofer® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Vifor S.A., Switzerland), in treatment of patients with mild and moderate iron-deficiency anaemia.
Protocol No.	No. TE_005_FER_CHT
Study phase	Phase III
Study Sponsor	Sandoz CJSC, Russia
Country	Russian Federation
Study drug	**Ferrum Lek®** (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia)
Marketing authorization status of the study drug	The drug product is registered in the Russian Federation.
Reference product	**MALTOFER®** (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Vifor S.A., Switzerland)
Marketing authorization status of the reference product	The drug is registered for human use in the Russian Federation.
Indication	Iron-deficiency anemia
Study purpose	The purpose of this study is to evaluate non-inferiority and safety of Ferrum Lek® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia), compared to MALTOFER® (Vifor S.A., Switzerland), in the treatment of patients with mild and moderate iron-deficiency anaemia.
Study objectives	**Main Objective:** To assess the non-inferiority of the therapeutic efficacy of Ferrum Lek® 2 tablets daily (200 mg), via assessing the effect on hemoglobin level in the blood (g/l) after 12 weeks of treatment for patients with iron-deficiency anemia of mild and moderate severity compared with MALTOFER® 2 tablets daily (200 mg) during the same period. **Secondary objectives:** Assessment of safety of Ferrum Lek® 2 tablets daily (200 mg), compared with MALTOFER® 2 tablets daily (200 mg), via the frequency, characteristics, intensity and relationship to treatment received of AEs.
Number of study sites	20 sites in the Russian Federation.

Number of subjects	It is planned to randomize 336 patients (168 patients in each treatment group) considering that no more than 15% of them will drop out before the end of study per protocol. Randomization will be stratified by two factors: - Gender (male vs female); - Hb level (80–94 g/L vs 95–110 g/L) Thus, it is necessary to receive data from at least 286 patients (143 patients in each treatment group) who completed the study according to protocol.
Duration of the study	Subject enrollment – not longer than 9 months. Treatment – 3 months (12 weeks). Follow-up visit (by phone) – 14 days after the last planned visit to the study cite. Total study duration – not more than 12 months.
Study design	A phase III, open-label, active-controlled, randomized, multicenter, prospective, comparative, parallel-group study (in the Russian Federation). Study population – 336 adult outpatients of both sexes with diagnosed mild and moderate iron-deficiency anemia (hemoglobin level below 110 g/L (in men and women), but above 80 g/L), who are iron-naive patients. Up to 480 subjects will be screened in order to achieve 336 randomizations of patients that meet inclusion/exclusion criteria per protocol. Upon signing of the informed consent for participation in the study, the subjects undergo screening for up to 7 days. The subjects who meet entry criteria will be randomized into two treatment groups at the ratio 1:1: The subjects in the **first group** (168 subjects; study drug) will receive Ferrum Lek® chewable tablets – 2 tablets daily (200 mg), during or right after meal; the daily dose administered once daily. The subjects in the **second group** (168 subjects; reference drug) will receive MALTOFER® chewable tablets – 2 tablets daily (200 mg), during or right after meal; the daily dose administered once daily. The subjects will take the drugs daily for 12 weeks and keep a Diary, where they will record administration of the study drugs and the concomitant therapy. Complete blood count and blood chemistry will be performed on all subjects. During the treatment period three visits are planned: I. Week 4: Day 29±2 from the therapy start, II. Week 8: Day 57±2 from the therapy start, III. Week 12: Day 84±2 final visit after therapy course completion. After the last planned visit to the study site, a Follow-up visit (by telephone) is to be made 14 days after the end of the active

	therapy period for the registration of delayed adverse events (98±2 days).
Inclusion criteria	1) The signed and dated written informed consent prior to participation in the study. 2) Men and women aged 18 and older (by the time of screening). 3) Outpatients. 4) Diagnosed iron-deficiency anemia, based on two criteria: a) hemoglobin level below 110 g/L (in men and women), but above 80 g/L, b) serum ferritin levels below 30 μg/L. 5) Women are eligible for enrollment if they are: **a)** **not fertile** (i.e., women in postmenopause or after surgical sterilization). Surgically sterile women are considered as female subjects with documented hysterectomy and/or bilateral ovariectomy and/or tubal ligation. Women in postmenopause are considered as women with menostasia for more than 1 year, with the corresponding clinical profile, e.g. older than 45 years, in absence of hormonal replacement therapy. However, in case of doubt, a blood sample shall be taken, where FSH content shall be above 40 IU/ml, and estradiol content – below 40 pg/ml (below 140 pmol/L), in order to confirm postmenopause. **OR** **b)** **fertile**, but the result of pregnancy test at screening is negative, and the subject agrees to use one of the following contraception methods constantly and properly (i.e. in accordance with the approved prescribing information and the doctor's orders) during the whole period of participation in the study: i) Total sexual abstinence ii) Oral contraceptives (combination drugs containing progestogen, or progestogen alone) iii) Injectable progestogen iv) Levonorgestrel implants v) Estrogen-containing vaginal ring vi) Transdermal contraceptive patches vii) Intrauterine device or intrauterine system viii) A male partner has been sterile (vasectomy with documented azoospermia) **prior to enrollment of a woman**, provided that he is the only partner of the female subject. For the purpose of this definition, "documented" is related to the result of a subject's medical examination by an investigator/responsible person or a subject's past medical history review for assessment of eligibility for enrollment, obtained during the interview with a subject or from his/her medical records. ix) Double barrier method: a condom or an occlusive cap (diaphragm or cervical/vault caps) with a spermicide (foam/gel/film/cream/suppository).
Exclusion criteria	1) Administration of any iron-containing drugs during the last 3 months. 2) History of erythropoietin drugs administration. 3) Hypersensitivity to iron therapy (both Oral and/or IV administration) and other components of the study drugs. 4) Hormone therapy (including the use of androgens/anabolic steroids) or administration of drugs that inhibit blood formation, less than 3 months before the start of the study. 5) History of severe allergic reactions or drug intolerance.

	6) Fructose intolerance, glucose-galactose malabsorption syndrome, and sucrase-isomaltase deficiency. 7) Pregnant or lactating women, or women intending to become pregnant during the study. 8) Failure of iron therapy for iron-deficiency anaemia in a subject's past medical history. 9) Heme metabolism disorders (e.g., sideroachrestic anaemia, lead anaemia, thalassaemia). 10) Iron overload including haemochromatosis and hemosiderosis 11) Other causes of anemia, apart from iron deficiency, including: a) Haemolysis (determined as per analyses results at screening, or as per anamnestic data), b) Vitamin B12 and folic acid deficiency (as per the screening data), c) Chronic kidney disease (creatinine clearance at screening is below 90 ml/min (based on Cockcroft-Gault Formula)), d) Systemic connective tissue diseases, chronic infectious diseases requiring regular therapy (as per the past medical history), and other conditions which may, in the investigator's opinion, be accompanied by anaemia of chronic diseases. 12) Dysfunction of the thyroid gland (based on the data obtained at screening). 13) Laboratory and clinical signs of an active inflammatory process for 10 days before screening. 14) AST, ALT, and total bilirubin levels exceeding the upper limit of normal 1.5 times and more. 15) Clinically apparent hypothyroidism, in the investigator's opinion. 16) Malignant diseases, including blood and lymphoid tissue disorders (leukemia, Hodgkin disease, myelodysplastic syndrome, myeloma, etc.) at screening or in the past medical history, provided that the remission was less than 5 years before screening. 17) Signs of bone marrow aplasia at screening or history of bone marrow aplasia. 18) The necessity of parenteral iron therapy, i.e. the following cases: a) impaired absorption in case of an intestinal pathology (enteritis, coeliac disease, malabsorption, small intestinal resection, stomach resection, including the duodenum); b) exacerbation of gastric or duodenal ulcer; c) the necessity of quick iron saturation, e.g. in patients with iron-deficiency anaemia with upcoming surgery; d) continuous vast blood loss and other causes, at the discretion of the investigator. 19) Known presence of an active infection caused by *Helicobacter pylori.* In case of presence of *Helicobacter pylori,* a subject may be enrolled after eradicative therapy. 20) Concomitant diseases and conditions, which, in the investigator's opinion, pose risk to a subject's safety in case of his/her participation in the study, or able to affect the safety data analysis

	in case of exacerbation of this disease/condition during the study, including: a) Myocardial infarction or stroke within 6 months before screening. b) Unstable angina; c) Severe arrhythmia, not controlled by drug therapy; d) Decompensated diabetes mellitus; e) Nephrological disorders; f) Other significant diseases, at the discretion of the investigator. 21) HIV infection (as per the screening data or the results of analysis performed within 6 months before screening). 22) Known or suspected drug or alcohol abuse for the last 2 years. 23) Suspected poor adherence of a subject (e.g., due to mental disorders). 24) Participation in any clinical drug studies less than 3 months before the study. 25) Blood donation / blood transfusion within 30 days prior to screening or planned blood transfusion at time of screening. 26) History of smoking, unless leave off smoking > 6 months.
Study endpoints:	**Primary endpoint:** • Changes in blood haemoglobin level (g/L) after 12-weeks of iron-deficiency anaemia treatment, a non-inferiority comparison, as compared with the baseline value (screening visit) between Ferrum Lek® and MALTOFER® groups.
	Secondary endpoints: • Absolute values and changes of blood hemoglobin level (g/L) after 4, 8 and 12-weeks of treatment in the study groups. • Changes in average values of iron metabolism parameters (ferritin, transferrin, percent transferrin saturation, serum iron) after 4, 8 and 12 weeks of iron-deficiency anaemia treatment, as compared with the baseline value in the study groups. • The frequency of response to therapy, determined as an increase in haemoglobin level by 20 g/L and more after 12-weeks of iron-deficiency anemia treatment in the study groups. • Frequency and severity of all adverse events observed during the study therapy in the study groups.
Examinations	Baseline data (demography, past medical history and laboratory data) will be collected for all subjects at screening. The parameters for the study therapy **efficacy evaluation** will be collected prior to treatment and after 12-weeks of treatment: • Haemoglobin (complete blood count), • Iron metabolism parameters (serum ferritin, serum iron, transferrin, percent transferrin saturation). **Safety evaluation** of the study therapy will be performed throughout the study and will be based on detection of any adverse events occurring during the study. The following will be performed:

	• Physical examination, including BP and heart rate measurement, and identification of complaints on digestive system disorders (at screening, during Visit 2 and the final visit). • Complete blood count (at screening and after 12-weeks of treatment), • Blood chemistry (at screening), • Urinalysis, pregnancy test (at screening), • Concomitant therapy recording (during each visit).
Statistical analysis	**Efficacy evaluation:** **Primary efficacy endpoint:** • Changes in blood haemoglobin level (g/L) after 12-weeks of iron-deficiency anaemia treatment (during the final visit), as compared with the baseline value (the screening visit) between the study groups. Analysis of covariance (ANCOVA) will be used as the main method of assessment of the primary endpoint, with baseline haemoglobin value being a covariate and four fixed factors (treatment group, gender, weight and baseline Hb level (80–94 g/L vs 95–110 g/L). Unrestricted least significant differences (LSD) method will be applied to the ANCOVA results with the calculation of least-square (LS) means with 95% CI for LS means for the difference between the study groups. Non-inferiority will be confirmed if the upper limit of the two-sided 95% confidence interval for LS mean will not exceed the pre-defined non-inferiority margin of 5 g/L. **Secondary efficacy endpoints:** All secondary efficacy analysis will be considered explorative, therefore, no multiplicity correction will be done for any tests, and no formal power calculation will be provided for the test results. • Absolute values and changes (compared to baseline) of blood hemoglobin level (g/L) after 12 weeks of treatment (during the final visit) between the study groups. Values at individual time points and values of change between the individual time points will be compared using the t-test or Mann-Whitney test depending on the normality of the distribution (which will be tested using Shapiro-Wilk test). Additionally, it is planned to use ANCOVA for analysis of changes in the assessed parameters over time where group will be a fixed factor, screening hemoglobin level will be a covariate , and hemoglobin level at Week 12 will be a response variable. • Changes in average values of iron metabolism parameters (ferritin, transferrin, percent transferrin saturation, serum iron) during the treatment period (from screening till the final visit) in the study groups. Values at individual time points (baseline and Week 12) and values of change between the individual time points (baseline and Week 12) will be compared using the t-test or Mann-Whitney test depending on

	the normality of the distribution (which will be tested using Shapiro-Wilk test). • The percentage (%) of subjects with response to the therapy, determined as an increase in hemoglobin level by 20 g/L and more after 12-weeks of treatment (from screening till the final visit) in the study groups. Comparison between the two groups will be performed using the Fisher's exact test or chi-square test depending on the number of expected observations per cell (<5 or ≥5). Two-sided 95% confidence interval will be calculated using exact Clopper-Pearson method. These changes will be compared between the groups using the t-test or Mann-Whitney test depending on the normality of the distribution (which will be tested using Shapiro-Wilk test). **Safety evaluation:** Safety evaluation will include: 1)determination of the total number, frequency and severity of: I. adverse events (AEs) irrespective of their relation to treatment; II. AEs related or possibly related to the treatment received; III. AEs requiring termination of the therapy. 2) Complaints on digestive system disorders (defined through MedDRA SMQ for the corresponding symptoms), considered as related to the study therapy by the investigator, will be analyzed separately. Descriptive statistics will be used for presentation of the result. Comparison of all AE incidence between the study groups will be performed using the Fisher's exact test or chi-square test depending on the number of expected observations per cell (<5 or ≥5), and comparison of intensity (and possible causal relationship between the treatment received and the AE) will be performed using Cochrane-Armitage trend test for the ordered categorical data. 3) Changes in laboratory test results (assessed in central laboratory) over time and frequency of abnormal test results (according to the normal ranges of central laboratory) will be summarized by group and also compared by group using corresponding tests for quantitative data and qualitative data (see below). 4) Changes in vital signs over time and frequency of abnormal physical examination findings will be summarized by group and also compared by group using corresponding tests for quantitative data and qualitative data (see below). Quantitative data will be compared using the t-test or Mann-Whitney test depending on the normality of the distribution (which will be tested using Shapiro-Wilk test). Qualitative data will be compared using the Fisher's exact test or chi-square test depending on the number of expected observations per cell (<5 or ≥5).
Interim analysis	One formal Interim analysis to assess the primary and secondary endpoints of efficacy is planned when the population of patients with

	available primary efficacy endpoint data is at least 240 patients (120 patients in each group treatment: the main group is Ferrum Lek® (iron (III) hydroxide polymaltosate) and the reference group MALTOFER® (iron (III) hydroxide polymaltosate). Safety and Efficacy will be assessed for all patients who received at least one dose of the randomized treatment at the time of the interim analysis. Based on the results of the interim analysis of the primary and secondary statistical endpoints of the efficacy and safety of the study Sponsor may decide to stop the study if study endpoints have been met as per protocol based on the results of interim analyses or sample size might be recalculated using results of interim analyses.
Justification of the sample size	Calculation of the sample size is based on published works focusing on iron (III) hydroxide polymaltosate efficacy: 1) Maltofer Product Information leaflet (http://www.aspenpharma.com.au/product_info/pi/PI_Maltofer.pdf) 2) Santiago P. Ferrous versus iron oral iron formulations for the treatment of iron deficiency: a clinical overview. Scientific World Journal. 2012;2012:846824. 3) Geisser P. Safety and efficacy of iron(III)-hydroxide polymaltose complex / a review of over 25 years' experience. Arzneimittelforschung. 2007;57(6A):439-52; 4) Toblli JE, Brignoli R. Iron(III)-hydroxide polymaltose complex in iron deficiency anemia / review and meta-analysis. Arzneimittelforschung. 2007;57(6A):431-8); 5) Reinisch W, Staun M, Tandon RK, Altorjay I, Thillainayagam AV, Gratzer C, Nijhawan S, Thomsen LL. A randomized, open-label, non-inferiority study of intravenous iron isomaltoside 1,000 (Monofer) compared with oral iron for treatment of anemia in IBD (PROCEED). Am J Gastroenterol. 2013 Dec;108(12):1877-88. 6) Zaim M, Piselli L, Fioravanti P, Kanony-Truc C. Efficacy and tolerability of a prolonged release ferrous sulphate formulation in iron deficiency anaemia: a non-inferiority controlled trial. Eur J Nutr. 2012 Mar;51(2):221-9. Based on the data of these publications, during 12-weeks of oral administration of iron (III) hydroxide polymaltosate the hemoglobin level can increase on average by 8-15 g/L with a standard deviation up to 15 g/L. As similar efficacy is expected in both groups, zero difference in primary efficacy parameter will be used for calculation

	of sample size. Maximum value of standard deviation was used – 15 g/L to take a maximum variability of the primary endpoint variable into account for properly powered study. The value of 5 g/L was selected as the non-inferior efficacy limit (in accordance with the limit stated and justified in the above mentioned works. Thus, the following assumptions were proposed for calculations: 1) Changes in blood hemoglobin level (g/L) after 12-weeks of iron-deficiency anemia treatment (during the final visit), as compared with the baseline value (the screening visit 0) are expected to be similar for the investigational and reference drug groups (i.e. expected difference between the two groups is set to zero). 2) The pooled standard deviation for the changes in hemoglobin level was 15 g/L. 3) Non-inferiority margin is 5 g/L. 4) The significance level (two-sided, in accordance with FDA recommendations) is 95%, which corresponds to the type I one-sided error 0.05. 5) Power of the study is 80%, which corresponds to the type II error 0.20. 6) Statistical hypotheses – evidence of a non-inferior efficacy: $H_0: \mu_A - \mu_B \leq 5.0$ $H_1: \mu_A - \mu_B > 5.0$ 7) The ratio between the study and control group sizes is 1:1 8) Interim analysis will be performed at 70% of the total sample size 9) O'Brien-Fleming alpha-spending function will be used with 80% power for efficacy boundary that will correspond to the following critical values for t-test: 2,628 for interim analysis (corresponds to p=0,005) and 1,976 for final analysis (corresponds to p= 0,0245); Calculation was performed using the clinfun package of Microsoft R Open software (available at https://mran.microsoft.com – Microsoft R Application network), that uses the group sequential design approach for the calculation of fixed sample sizes for the interim and final analysis. According to the output from the clinfun package, 143 subjects per group (286 subjects in total) should complete the study in order to

	confirm non-inferiority. 200 (100 per group) subjects should have evaluable data to perform interim analysis. Taking 15% withdrawal rate into account, one need to randomize 336 subjects (168 subjects per group). Taking 30% screening failure rate, up to 480 subjects should be screened to achieve target randomization.

1 General information

1.1 Protocol name, Protocol ID, and date:

Name: A multicenter, open-label, randomized, active-controlled study to evaluate the efficacy and safety of Ferrum Lek® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia), as compared with Maltofer® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Vifor S.A., Switzerland), in subjects with mild and moderate iron deficiency anemia.
ID: TE_005_FER_CHT
Protocol version: 2.0
Date: March 20, 2019

1.2 Study Sponsor:

Study Sponsor:

Name of organization:	Sandoz CJSC, Russia
Address:	72 Leningradsky Prospekt, bld. 3, *125317* Moscow, *Russia*
Telephone:	[redacted]
E-mail:	sandoz.russia@sandoz.com

Sponsor's representative:

[redacted]	Full name: [redacted] Phone: [redacted] Fax: [redacted] E-mail: [redacted]
[redacted] **Novartis Pharma** [redacted]	Full name: [redacted] Address: [redacted] Phone: [redacted] Mobile: [redacted] (24h) Fax: [redacted] E-mail: [redacted]

1.3 Sponsor's representative for the medical questions:

Full name:	[redacted]
Position:	[redacted]

Name of organization:	Sandoz CJSC, Russia
Address:	*72 Leningradsky Prospekt, bld. 3, 125317* Moscow, Russia
Telephone:	Phone: [redacted] Fax: [redacted]
E-mail:	[redacted]

1.4 Sponsor's representative for the medical questions (back up):

Full name: Position:	[redacted]
Name of organization:	Sandoz CJSC, Russia
Address:	72 Leningradsky Prospekt, bld. 3, 125317 Moscow, Russia
Telephone:	T.: [redacted] ext [redacted] Fax: [redacted] Mobile: [redacted]
E-mail:	[redacted]

1.5 Study Monitor:

Contract research organization (CRO) responsible for study conduct

[redacted]
Address: [redacted]
T.: [redacted]; Fax: [redacted]
Email address [redacted]

1.6 Clinical laboratories and other organizations engaged in the study

Name of organization:	[redacted]
Address:	[redacted]
Telephone:	[redacted]
E-mail:	[redacted]

2 Study rationale

2.1 Study drugs

2.1.1 Study drug: Ferrum Lek®

Trade name: Ferrum Lek®
International non-proprietary name or generic name: iron [III] hydroxide polymaltosate.
Chemical name: iron [III] hydroxide polymaltosate
Dosage Form: chewable tablets
Composition:
1 chewable tablet contains:
Active substance: iron (III) hydroxide polymaltosate 400 mg (equivalent to 100 mg of iron).
Excipients: macrogol 6000 — 37.0 mg; aspartame — 1.5 mg; chocolate flavoring — 0.6 mg; talc — 21.0 mg; dextrates to 730.0 mg.
Appearance:
Dark brown, round, flat tablets with light brown impregnations and a bevel edge.
Pharmacotherapeutic group: iron drug.
ATC code: B03AB05.
Pharmacodynamic properties
Pharmacodynamics
Polynuclear iron (III) hydroxide is surrounded by a number of covalently bound polymaltose molecules from the outside in iron (III) hydroxide polymaltosate, which gives a total average molecular weight of about 50 kDa.
The polynuclear iron (III) hydroxide polymaltosate core has a structure similar to that of the core of the physiological iron storage protein ferritin. Iron (III) hydroxide polymaltosate is stable and under physiological conditions does not emit a large number of iron ions. Due to its size, iron (III) hydroxide polymaltosate diffusion degree through the mucosa is approximately 40 times less than that of the iron (II) hexahydrate complex. Iron from iron (III) hydroxide polymaltosate is actively absorbed in the intestine.
The efficacy of iron (III) hydroxide polymaltosate for normalizing the hemoglobin content and iron pool replenishment was demonstrated in numerous randomized controlled clinical studies using placebo or an active comparator in adults and children with different iron pool status.
Pharmacokinetics
Absorption
Iron from iron (III) hydroxide polymaltosate is absorbed in accordance with the controlled mechanism. The increase in serum iron content after the drug administration does not correlate with total iron absorption, measured as incorporation into hemoglobin (Hb). Studies with a labeled radioisotope of iron (III) hydroxide polymaltosate revealed a strong correlation between the inclusion of iron in erythrocytes and the iron content throughout the body. The maximum activity of iron absorption from iron (III) hydroxide polymaltosate occurs in the duodenum and small intestine. As in case of other oral iron drugs, the relative absorption of iron from iron (III) hydroxide polymaltosate defined as incorporation into hemoglobin, decreases with increasing doses of iron. In addition, a correlation was observed between the degree of iron deficiency (in particular serum ferritin concentration) and the relative amount of absorbed iron (i.e., the greater the iron deficiency, the better the relative absorption). In subjects with anemia, absorption of iron from iron (III) hydroxide polymaltosate, in contrast to iron salts, increased in the presence of food.

Distribution
Distribution of iron from iron (III) hydroxide polymaltosate after absorption was studied in a study using the technique of double isotopes (^{55}Fe and ^{59}Fe).
Biotransformation
The absorbed iron binds to transferrin and is used to synthesize hemoglobin in the bone marrow or is stored, mainly in the liver, where it binds to ferritin.
Excretion
Non-absorbed iron is excreted by the intestine (with feces).

Therapeutic indications

- treatment of latent iron deficiency (iron deficiency without anemia);
- treatment of symptomatic iron deficiency anemia;
- increased need for iron during pregnancy and lactation, blood donation, intensive growth, vegetarians and the elderly.

Contraindications

- iron overload (e.g., hemosiderosis, hemochromatosis);
- impairment of iron utilization (e.g., lead anemia, sideroachrestic anemia, thalassemia);
- anemia other than iron deficiency anemia (e.g., haemolytic anemia, megaloplastic anemia due to vitamin B12 deficiency);
- children under 12 years old (due to the need to prescribe small doses in this age group it is recommended to use Ferrum Lek® syrup 10 mg/ml).

Administration during pregnancy and lactation
Pregnancy
Until now, there have been no reports of serious adverse reactions after oral administration of iron (III) hydroxide polymaltosate at therapeutic doses for the treatment of anemia during pregnancy. The data obtained from animal studies showed no danger to the fetus or the mother. There is no clinical data on the use of Ferrum Lek® in the first trimester of pregnancy.
The studies conducted in pregnant women after the end of the first trimester of pregnancy revealed no adverse effects of iron (III) hydroxide polymaltosate in mothers and/or newborns. Consequently, adverse effect on the fetus is unlikely following the administration of iron (III) hydroxide polymaltosate.
Lactation
Woman breast milk contains iron bound to lactoferrin. The amount of iron that passes from iron (III) hydroxide polymaltosate to breast milk is unknown. It is unlikely that the administration of iron (III) hydroxide polymaltosate in lactating women can lead to adverse effects in the child. As a precaution to women of childbearing age and women during pregnancy and lactation, Ferrum Lek® should be administered only after consultation with the doctor. The benefit/risk ratio should be evaluated.

Posology and method of administration
Oral dosage form.
The daily dose can be divided into several doses or taken at a time. Ferrum Lek® should be administered during or immediately after meals. Ferrum Lek®, 100 mg chewable tablets, can be chewed or swallowed whole.
The daily dose of the drug depends on the degree of iron deficiency (see table of daily doses).

Table of daily doses

Patient populations	Treatment of iron deficiency anemia	Treatment of iron deficiency without anemia	Increased iron requirement
Children older than 12 years, adults and lactating women	1–3 chewable tablets (100–300 mg of iron)	1 chewable tablet (50–100 mg of iron)	--
Pregnant women	2–3 chewable tablets (200–300 mg of iron)	1 chewable tablet (100 mg of iron)	1 chewable tablet (100 mg of iron)

Treatment of iron deficiency anemia in children older than 12 years and adults
100 to 300 mg of iron (1–3 tablets) per day for 3–5 months until the normal hemoglobin (Hb) level is reached. After this, treatment should be continued for several weeks at the dose described for iron deficiency without anemia in order to replenish iron stores.
Treatment of iron deficiency anemia during pregnancy
200 to 300 mg of iron (2–3 tablets) per day until the normal hemoglobin (Hb) level is reached. After this, treatment should be continued at least until the end of pregnancy at a dose described for iron deficiency without anemia in order to replenish iron stores and meet the increased requirements for iron due to pregnancy.
Treatment and prevention of iron deficiency without anemia in children older than 12 years and adults
100 mg (1 tablet) per day for 1–2 months.
Paediatric use
Ferrum Lek® 100 mg chewable tablets are contraindicated for children under 12 years old.
Side effects
Ferrum Lek® is generally well tolerated. Side effects are mainly mild and transient.
Side effects are mainly mild and transient. According to the World Health Organization (WHO), adverse reactions are classified depending on their incidence as follows: very common (> 1/10), common (> 1/100, < 1/10), uncommon (> 1/1,000, < 1/100), rare (> 1/10,000, < 1/1,000), very rare (< 1/10,000), not known (cannot be estimated using the available data).
Nervous system disorders
uncommon: headache.
Gastrointestinal disorders
very common: feces discolored (in 23% of patients, this is a known adverse reaction to iron drugs);
common: diarrhea, nausea, indigestion;
uncommon: vomiting, constipation, abdominal pain, discoloration of tooth enamel (in 0.6% of patients, this is a known adverse reaction to iron drugs);
Skin and subcutaneous tissue disorders
uncommon: rash, including exanthema, itching.
Overdose
In case of an overdose of Ferrum Lek®, iron overload or intoxication is unlikely due to the low toxicity of iron (III) hydroxide polymaltosate and controlled iron uptake. No cases of fatal unintentional poisoning were reported.
Interaction with other medicinal products
Interactions of iron (III) hydroxide polymaltosate with tetracycline or aluminum hydroxide have been studied. No significant decrease in tetracycline absorption has been observed. Plasma concentrations of tetracycline did not drop below the effective level. Absorption of iron from iron (III) hydroxide polymaltosate did not decrease under the influence of aluminum hydroxide or tetracycline. Thus, concomitant administration of iron (III) hydroxide polymaltosate with

tetracycline and other phenolic compounds, as well as aluminum hydroxide, is allowed. The studies in rats using tetracycline, aluminum hydroxide, acetylsalicylic acid, sulfasalazine, calcium carbonate, calcium acetate and calcium phosphate in combination with vitamin D3, bromazepam, magnesium aspartate, D-penicillamine, methyldopa, paracetamol and auranofin revealed no interactions with iron (III) hydroxide polymaltosate.
No interaction of iron (III) hydroxide polymaltosate with food components such as phytic acid, oxalic acid, tannin, sodium alginate, choline and choline salts, vitamin A, vitamin D3, and vitamin E, soybean oil and soybean flour was observed either. These results indicate that iron (III) hydroxide polymaltosate can be administered during or immediately after meals.
The administration of the drug does not affect the results of the detection of hidden blood (with a selective determination of hemoglobin), therefore, treatment interruption is not necessary.
Concomitant use of parenteral and oral iron drugs should be avoided, since the absorption of iron taken orally slows down.

Special warnings

Ferrum Lek® is not expected to have an effect on the daily insulin requirement in patients with diabetes mellitus. 1 chewable tablet contains 0.04 bread units.
Anemia can be caused by infectious diseases or malignant neoplasms. Since iron can only be taken after the root cause of the disease has been eliminated, the benefit/risk ratio of treatment should be determined.
During treatment with Ferrum Lek®, dark discoloration of feces can be noted, but this is not clinically significant.

Effects on ability to drive and use machines

No data available. It is unlikely that Ferrum Lek® influences the ability to drive or use mechanisms.

Pharmaceutical form

100 mg chewable tablets.

Primary packaging
10 tablets in an Al/Al strip or in an Al/Al blister.
Secondary packing
3, 5 or 9 strips or blisters in a carton pack along with the Patient Information Leaflet.

Storage Conditions

At a temperature below 25 °C.
Keep out of reach of children.

Shelf life

5 years.
Do not use after the expiry date.

Pharmacy purchasing terms

By prescription.

Manufacturer

PL Holder: Sandox d.d. Verovskova 57, 1000 Ljubljana, Slovenia
Manufacturer: Lek d. d. Verovskova 57, 1526 Ljubljana, Slovenia

Send customer complaints to

Sandoz CJSC, 72 Leningradsky Prospekt, bld. 3, 125315 Moscow;
telephone: [redacted] fax: [redacted].

2.1.2 Reference product: MALTOFER®

Trade name: MALTOFER® (Maltofer®)
International non-proprietary name or generic name: iron [III] hydroxide polymaltosate

Chemical name: iron [III] hydroxide polymaltosate
Dosage Form: chewable tablets
Composition:
1 tablet contains:

active substance:	
iron [III] hydroxide polymaltosate	357.0 mg
equivalent to iron	100.0 mg
excipients:	
dextrates	232.0 mg
macrogol 6000	37.0 mg
purified talc	21.0 mg
sodium cyclamate	9.0 mg
vanillin	2.9 mg
cocoa powder	29.0 mg
chocolate flavor	0.6 mg
microcrystalline cellulose	up to 730.0 mg

Appearance
Dark brown, round, flat scored tablets with white impregnations.
Pharmacotherapeutic group: iron drug.
ATC code: B03AB05
Pharmacodynamic properties
Pharmacodynamics
Polynuclear iron (III) hydroxide is surrounded by a number of covalently bound polymaltose molecules from the outside in iron (III) hydroxide polymaltosate, which gives a total average molecular weight of about 50 kDa. The polynuclear iron (III) hydroxide polymaltosate core has a structure similar to that of the core of the physiological iron storage protein ferritin. Iron (III) hydroxide polymaltosate is stable and under physiological conditions does not emit a large number of iron ions. Due to its size, iron (III) hydroxide polymaltosate diffusion degree through the mucosa is approximately 40 times less than that of the iron (II) hexahydrate complex. Iron from iron (III) hydroxide polymaltosate is actively absorbed in the intestine. The efficacy of MALTOFER® for normalizing the hemoglobin content and iron pool replenishment was demonstrated in numerous randomized controlled clinical studies using placebo or an active comparator in adults and children with different iron pool status.
Pharmacokinetics
Absorption
Iron from iron (III) hydroxide polymaltosate is absorbed in accordance with the controlled mechanism. The increase in serum iron content after the drug administration does not correlate with total iron absorption, measured as incorporation into hemoglobin (Hb). Studies with a labeled radioisotope of iron (III) hydroxide polymaltosate revealed a strong correlation between the inclusion of iron in erythrocytes and the iron content throughout the body. The maximum activity of iron absorption from iron (III) hydroxide polymaltosate occurs in the duodenum and small intestine. As in case of other oral iron drugs, the relative absorption of iron from iron (III) hydroxide polymaltosate defined as incorporation into hemoglobin, decreases with increasing doses of iron. In addition, a correlation was observed between the degree of iron deficiency (in particular serum ferritin concentration) and the relative amount of absorbed iron (i.e., the greater the iron deficiency, the better the relative absorption). In subjects with anemia, absorption of iron from iron (III) hydroxide polymaltosate, in contrast to iron salts, increased in the presence of food.

Distribution
Distribution of iron from iron (III) hydroxide polymaltosate after absorption was studied in a study using the technique of double isotopes (^{55}Fe and ^{59}Fe).
Biotransformation
The absorbed iron binds to transferrin and is used to synthesize hemoglobin in the bone marrow or is stored, mainly in the liver, where it binds to ferritin.
Excretion
Non-absorbed iron is excreted by the intestine (with feces).

Therapeutic indications

- Treatment of iron deficiency without anemia (latent iron deficiency) and treatment of symptomatic iron deficiency anemia.
- Increased need for iron during pregnancy and lactation, blood donation, intensive growth, vegetarians and the elderly.

Contraindications

- The established hypersensitivity to iron (III) hydroxide polymaltosate or to any excipient.
- Iron overload (e.g., hemosiderosis, hemochromatosis).
- Impairment of iron utilization (e.g., lead anemia, sideroachrestic anemia, thalassemia).
- Anemia other than iron deficiency anemia (e.g., haemolytic anemia, megaloplastic anemia due to vitamin B12 deficiency).
- Children under 12 years old (due to the need to prescribe small doses in this age group it is recommended to use MALTOFER® 50 mg/ml oral drops, or MALTOFER® 10 mg/ml syrup).

Administration during pregnancy and lactation
Pregnancy
Until now, there have been no reports of serious adverse reactions after oral administration of MALTOFER® at therapeutic doses for the treatment of anemia during pregnancy. Animal data showed no
danger to the fetus or mother. There is no clinical data on the use of MALTOFER® in the first trimester of pregnancy.
The studies conducted in pregnant women after the end of the first trimester of pregnancy revealed no adverse effects of MALTOFER® in mothers and/or newborns. Consequently, adverse effect on the fetus is unlikely following the administration of MALTOFER®. Lactation
Woman breast milk contains iron bound to lactoferrin. The amount of iron that passes from iron (III) hydroxide polymaltosate to breast milk is unknown. It is unlikely that the administration of MALTOFER® in lactating women can lead to adverse effects in the child. As a precaution to women of childbearing age and women during pregnancy and lactation, MALTOFER® should be administered only after consultation with the doctor. The benefit/risk ratio should be evaluated.

Posology and method of administration
Oral dosage form.
The daily dose can be divided into several doses or taken at a time. MALTOFER® should be administered during or immediately after meals. MALTOFER®, 100 mg chewable tablets, can be chewed or swallowed whole. The daily dose of the drug depends on the degree of iron deficiency (see table of daily doses).

Table of daily doses

Patient populations	Treatment of iron deficiency anemia	Treatment of iron deficiency without anemia	Increased iron requirement
Children older than 12 years, adults and lactating women	1–3 tablets (100–300 mg of iron)	1 tablet (50–100 mg of iron)	*
Pregnant women	2–3 tablets (200–300 mg of iron)	1 tablet (100 mg of iron)	1 tablet (100 mg of iron)

*Due to the need to prescribe small doses according to these indications, it is recommended to use MALTOFER® 50 mg/ml oral drops, or MALTOFER® 10 mg/ml syrup).

Treatment of iron deficiency anemia in children older than 12 years and adults
100 to 300 mg of iron (1–3 tablets) per day for 3–5 months until the normal hemoglobin (Hb) level is reached. After this, treatment should be continued for several weeks at the dose described for iron deficiency without anemia in order to replenish iron stores.
Treatment of iron deficiency anemia during pregnancy
200 to 300 mg of iron (2–3 tablets) per day until the normal hemoglobin (Hb) level is reached. After this, treatment should be continued at least until the end of pregnancy at a dose described for iron deficiency without anemia in order to replenish iron stores and meet the increased requirements for iron due to pregnancy.
Treatment and prevention of iron deficiency without anemia in children older than 12 years and adults
100 mg (1 tablet) per day for 1–2 months. If a smaller dose is required for prevention, MALTOFER® 50 mg/ml oral drops, or MALTOFER® 10 mg/ml syrup can be used. Paediatric use MALTOFER® 100 mg chewable tablets are contraindicated for children under 12 years old. The dosage form and the concentration of MALTOFER® 50 mg/ml oral drops, and MALTOFER® 10 mg/ml syrup are mor suitable for taking the recommended dose in this age group.

Side effects

Safety and tolerability of MALTOFER® was evaluated in numerous clinical studies. The main adverse drug reactions (ADRs), noted in these studies, occurred in the following three classes of systems and organs.
Adverse drug reactions reported in clinical studies

System organ class	Very common (>1/10)	Common (> 1/100, < 1/10)	Uncommon (> 1/1000, < 1/100)
Nervous system disorders	--	--	Headache
Gastrointestinal disorders	Feces discolouration[1]	Diarrhea, nausea, indigestion	Vomiting, constipation, abdominal pain, discoloration of tooth enamel[2]
Skin and subcutaneous tissue disorders	--	--	Rash[3], pruritus

[1] Often registered as an adverse event (in 23% of patients), this is a well-known ADR for oral iron drugs.
[2] Registered as an adverse event in 0.6 % of patients, this is a well-known ADR for oral iron drugs.
[3] Including exanthema.

Spontaneous post-marketing reports about adverse drug reactions
No additional adverse drug reactions were reported.
Laboratory abnormalities
No data available.

Overdose
In case of an overdose of MALTOFER®, iron overload or intoxication is unlikely due to the low toxicity of iron (III) hydroxide polymaltosate and controlled iron uptake. No cases of fatal unintentional poisoning were reported.

Interaction with other medicinal products
Interactions of iron (III) hydroxide polymaltosate with tetracycline or aluminum hydroxide have been studied. No significant decrease in tetracycline absorption has been observed. Plasma concentrations of tetracycline did not drop below the effective level. Absorption of iron from iron (III) hydroxide polymaltosate did not decrease under the influence of aluminum hydroxide or tetracycline. Thus, concomitant administration of iron (III) hydroxide polymaltosate with tetracycline and other phenolic compounds, as well as aluminum hydroxide, is allowed. The studies in rats using tetracycline, aluminum hydroxide, acetylsalicylic acid, sulfasalazine, calcium carbonate, calcium acetate and calcium phosphate in combination with vitamin D3, bromazepam, magnesium aspartate, D-penicillamine, methyldopa, paracetamol and auranofin revealed no interactions with iron (III) hydroxide polymaltosate.
No interaction of iron (III) hydroxide polymaltosate with food components such as phytic acid, oxalic acid, tannin, sodium alginate, choline and choline salts, vitamin A, vitamin D3, and vitamin E, soybean oil and soybean flour was observed either. These results indicate that iron (III) hydroxide polymaltosate can be administered during or immediately after meals. The administration of the drug does not affect the results of the detection of hidden blood (with a selective determination of hemoglobin), therefore, treatment interruption is not necessary. Concomitant use of parenteral and oral iron drugs should be avoided, since the absorption of iron taken orally slows down.

Special warnings
MALTOFER® is not expected to have an effect on the daily insulin requirement in patients with diabetes mellitus. 1 chewable tablet contains 0.04 bread units.
Anemia can be caused by infectious diseases or malignant neoplasms. Since iron can only be taken after the root cause of the disease has been eliminated, the benefit/risk ratio of treatment should be determined.
During treatment with MALTOFER®, dark discoloration of feces can be noted, but this is not clinically significant.

Effects on ability to drive and use machines
No data available. It is unlikely that MALTOFER® influences the ability to drive or use mechanisms.

Pharmaceutical form
100 mg chewable tablets.
10 tablets in polyethylene laminated aluminum foil blisters. 1 or 3 blisters per carton pack along with the Patient Information Leaflet.

Storage Conditions
In a place protected from light at a temperature below 25°C. Keep out of reach of children.

Shelf life
5 years.
Do not use after the expiry date indicated on the package.

Pharmacy purchasing terms
By prescription.

Marketing authorization holder / company that performs release quality control
Vifor (International) Inc.
Rechenstrasse 37, CH-9014 St. Gallen, Switzerland
Vifor (International) Inc.
Rechenstrasse 37, CH-9014 St. Gallen, Switzerland
Manufacturer
Vifor S.A.
Route de Moncor 10, CH-1752 Villars-sur-Glane, Switzerland
Vifor S.A.
Route de Moncor 10, CH-1752 Villars-sur-Glane, Switzerland
Organization for customer complaints
Takeda Pharmaceutical Co., Ltd.
2, Usacheva st., bld. 1, 119048 Moscow
Tel. +7 (495) 933 55 11. Fax: +7 (495) 502 16 25.
E-mail: russia@takeda.com
Internet: www.takeda.com.ru

2.2 Summary of Non-Clinical Study Results

Introduction

This non-clinical review contains information regarding the composition of Ferrum Lek®, which is marketed the EU.

In order to provide brief and up-to-date information, this review is devoted to a medicinal product containing iron (III) hydroxide polymaltosate as the active substance.

In particular, the review will be based on data published in recent literature, taking into account any new information on the safety and efficacy of the drug.

According to the above provisions, non-clinical data of the active substance of the drug, iron (III) hydroxide polymaltosate, will be highlighted first of all. The next part will contain all reports and publications in accordance with the updated edition of November 2003 (the first edition was published in 1979) of the Uniform requirements for manuscripts submitted to biomedical journals of the International Committee of Medical Journal Editors (ICMJE).

In the sections printed in italics, a critical assessment of safety pharmacology will be presented as part of a comparison of non-clinical data and a number of well-known clinical indicators. In general, non-clinical studies showed that non-clinical pharmacokinetic and pharmacodynamic parameters are similar to those found in clinical studies.

2.2.1 Overview of the non-clinical study strategy

Iron is a natural component of the body, and it is necessary for the formation of hemoglobin and oxidative processes occurring in living tissues. It is a component of hemoglobin, myoglobin and a number of enzymes. A third part of the total iron in the body is stored in the form of ferritin and hemosiderin.

The body contains about 4 g of iron, most of which is contained in hemoglobin. Ionized iron is a component of certain enzymes necessary for energy transfer (such as cytochrome oxidase, xanthine oxidase, succinate dehydrogenase). Prescription of iron drugs allows you to correct hematopoietic disorders, associated with iron deficiency. However, iron does not activate hematopoiesis and does not eliminate structural changes in hemoglobin that are not associated with iron deficiency.

Iron is also not used to treat anemia, the cause of which is not associated with its deficiency. Iron can be bivalent or trivalent. Unlike Fe (II) ions, Fe (III) ions form a high-molecular-weight polynuclear Fe (III) hydroxide in a weakly acidic medium (at pH of about 2). Water-soluble complexes of polynuclear Fe (III) hydroxide polymaltose or polysomaltose are iron dextrin or iron dextran. In addition to parenteral administration, polynuclear iron(III) hydroxide complexes are also effective when administered orally [1].

Iron(III) hydroxide polymaltose complex (IPC) is an organic complex comprising trivalent iron, which is used as an antianemic food additive.

One chewable tablet contains 100 mg of iron in the form of iron (III) hydroxide polymaltose complex.

One teaspoon (5 ml) of syrup contains 50 mg of iron in the form of iron (III) hydroxide polymaltose complex.

Each ampoule of the solution for intramuscular injection (2 ml) contains 100 mg of iron in the form of iron polymaltosate, whereas each ampoule of the solution for intravenous injection (5 ml) contains 100 mg of iron in the form of iron sucrose.

The iron polymaltose complex for oral use is a water-soluble, non-ionized polynucleated iron hydroxide polymaltose complex with a molecular weight of more than 150.00.

This drug is intended to treat iron deficiency anemia (sideropenia) or blood loss.

2.2.2 Pharmacology

2.2.2.1 Primary pharmacodynamics

Iron is an integral component of hemoglobin formation in vivo, an adequate amount of which is required to maintain effective erythropoiesis, and the resulting ability of blood to carry oxygen. Iron contained in myoglobin demonstrated the same function. Iron is also a co-factor for a number of natural enzymes, including cytochromes involved in electron transport. Iron is essential for catecholamine metabolism and the adequate functioning of neutrophils. When administered with food or as an additive, iron passes through the cells of the mucosa in the glandular state in a complex with transferrin protein. Iron is transported in this form to the bone marrow in the body, where it participates in the formation of red blood cells. Iron participates in the formation of hemosiderin and ferritin in a protein-bound form, replenishes depleted body stores or incorporates into hemoglobin. The body iron store is divided between the natural iron-containing compounds and the excess iron stored in the tissues. Hemoglobin predominates among the natural iron-containing fractions. This protein with a molecular weight of 64,500 Da includes 4 iron atoms per molecule. About 73% of iron in the body is contained in hemoglobin, the circulation of which continuously occurs in the body as red blood cells form. Of the total amount of iron in the body, 12–17% is stored in the form of two molecules, ferritin and hemosiderin, each of which can bind a large number of iron atoms. Ferritin is a protein the function of which is to deposit iron. It can exist in the form of individual molecules or form aggregates. Iron can occupy more than 30% of the ferritin mass (4000 iron atoms per ferritin molecule). Ferritin in the aggregated form, known as hemosiderin, contains about one third of the total amount of iron in normal condition and becomes visible under light microscopy. The predominant sites of iron deposition are the reticuloendothelial system and hepatocytes, despite the fact that a part of the iron is stored in the muscles. Apoferritin has a molecular weight of about 450,000 Da and includes 24 polypeptide subunits, inside of which there is a cavity for storing polynuclear phosphorylated iron hydroxide. Myoglobin contains 5 to 15% of iron, and acts as an oxygen reservoir for muscle cells [2,3]
Other forms of natural iron include various iron-dependent and non-gem-containing enzymes.
Small but highly significant amounts of iron (0.2%) are represented in the body in a transferrin-bound form. The internal charge of iron is compensated by a plasma protein, known as transferrin. This β1-glycoprotein has a molecular weight of about 76,000 Da [4]
Iron is transported from transferrin into the cells via specific plasma membrane transferrin receptors. Iron transferrin complexes bind to receptors, resulting in a tertiary complex, which is captured by receptor-mediated endocytosis. After that, iron dissociates in an pH-dependent mechanism in the acid vesicular compartment inside the cell, and the receptors return apotransferrin to the cell surface, from which it is released into the extracellular space [5].
When the body is saturated with iron, the synthesis of transferrin receptors slows down, and the production of transferrin is activated. In case of iron deficiency, the cells express more transferrin receptors, and the concentration of ferritin is reduced, which allows to maximize the level of iron absorption by cells and prevent its deposition. Isolation of human transferrin receptor genes and ferritin provided an opportunity to further study the molecular basis of the regulation of the described processes. Lactoferrin, a component of breast milk, mucosal tissues and white blood cells, or leukocytes, also binds some amount of iron in the body. Small amounts of iron, not included in the listed compounds, are included in a large number of enzymes involved in metabolism. These include oxidases, catalases, reductases, peroxidases and dehydrogenases. Each enzyme has the important function of electron reversing donor or acceptor during cellular metabolism. Iron-sensitive binding elements control the rate of translation and the stability of messenger RNA, which encodes ferritin and transferrin receptor [6].

Absorption is considered to be regulated by a single hematopoietic transcription factor (NF-E2), which binds intestinal transport and erythropoiesis by global control of genes in chromosomes [6,7]
A lot of studies investigated the often expressed opinion that iron (II) is more efficiently absorbed and utilized following oral administration in comparison with iron (III).
As a result, it was concluded that iron (II) and iron (III) do not differ in terms of therapeutic efficacy.

In vivo studies

Bioavailability of iron valence forms

Rats

No difference in the bioavailability of iron forms of different valencies was observed in experiments on animals. A prerequisite was the administration of iron in the ionized state. The results were confirmed by in vivo studies in healthy and iron deficient rats after intragastric administration of radioactively labeled ^{59}Fe (FeSO4), ^{59}Fe ($FeCl_3$) and ^{59}Fe-(Fe (III)-polymaltose) organic complex. Utilization of iron obtained in the form of an organic complex was somewhat less active in iron-deficient rats than in animals treated with FeSO4. In addition to detecting ^{59}Fe-radioactivity 6 days after the administration of the drug, the level of ^{59}Fe-radioactivity in the blood of iron-deficient rats was higher than that detected in healthy animals. The proportion of radioactivity present in the liver, in contrast, was higher in healthy animals compared with iron-deficient rats. In other words, this means that the ^{59}Fe-labeled iron of all three compounds studied is equivalently utilized.
Experiments were also conducted in healthy and iron-deficient rats that received food containing small amounts of iron. The animals were taken several samples for blood test. The hemoglobin content in the blood of healthy rats ranged 12 to 14 g/dL, and in iron deficient rats it was 6 to 8 g/dL. Iron was injected with a probe into the stomach, and traces of radioactivity of compounds labeled with ^{59}Fe-ion were detected on day 6 after the administration of the drug. No differences in the absorption level of divalent and trivalent ions were detected provided that iron was used in the ionized state. A similar effect can be achieved while maintaining pH of the prescribed solution at a level of less than 2.5. The administration of two- or trivalent ionized iron with food to healthy rats led to a slight decrease in the amount of iron absorbed. This difference disappeared after the administration of ionized iron with food to iron-deficient animals. The level of iron accumulation in the body of rats was investigated after its simultaneous administration with a mixture of amino acids and increasing amounts of HPO_{42} anions. While the level of absorption of iron iron in healthy animals decreased slightly, the accumulation activity of divalent ions in the body has increased dramatically [9].

Pigs

When testing these drugs in the treatment of iron deficiency or iron deficiency anemia in the models of anemia or sideropenia in 4-day piglets, the following results were obtained. Studies performed in newborn piglets can be compared with clinical data, as piglets manage to create typical models of anemia, most similar to the pathophysiological pattern observed in humans. The therapeutic result was evaluated on the basis of the analysis of the following parameters: serum iron content, iron total binding capacity (TBC), hemoglobin, hematocrit, the number and diameter of red blood cells. The equivalent efficacy of both groups of drugs was confirmed [10].

Bioavailability of iron contained in salts and complexes

Rats

A comparison of the absorption level was carried out in mature rats which were orally administered iron at doses of 5, 25, 50, and 250 μg. The effect of activated erythropoiesis was studied in 50 and 250 μg dose groups after sampling of 20 ml of blood by venesection two weeks before the beginning of the tests. The comparative bioavailability of iron sulfate and iron polymaltose complex was evaluated in hemoglobin synthesis processes. Iron from the salt and the complex was

equally accessible for hemoglobin synthesis in rats after venesection, administered a dose of 250 μg (41.2 and 35.6%) ($0.7 < p < 0.6$). In these bioavailability studies in rats, the absorption of doses of 5 and 25 μg was evident for salt. However, the differences disappeared after the preliminary erythropoiesis stimulation by phlebotomy, as a result of which a dose of 250 μg of iron was absorbed with equal efficiency both from the polymaltose complex and ferrous sulfate. Such differences are inexplicable, and since salts and high-molecular complexes can use different transport routes in enterocytes, further studies are required to study these mechanisms [11]

Radioactively marked ferrous sulfate and an iron hydroxide polymaltose complex were used to study the mechanisms of iron utilization after oral administration to healthy rats and anemic rats. Measurements of the radioactivity of blood serum, compact red cells, whole blood, liver, kidneys, spleen, bone tissue and, in some cases, gastrointestinal tract tissues were performed for doses of 0.84 to 41.9 mg Fe/kg. No significant differences in bioavailability and ways of iron utilization between the two studied drugs were observed. Differences in pharmacokinetic behavior after oral administration, especially in non-anemic rats, were confirmed by demonstrating the same level of increase in serum iron content after 10–20 times lower dose of $FeSO_4$ in comparison with the iron-polymaltose complex. A significant difference between the two studied drugs is the kinetics of serum release and excretion, especially in non-anemic rats. Also, they have a different volume of distribution and clearance [12].

A comparative bioavailability study of the correlated amounts of iron ascorbate and iron polymaltose complex was also carried out in rats. The studies were conducted in healthy animals under standard conditions or with insufficient or excessive iron content, which was achieved by manipulation with its stores or erythropoietic activity. No significant differences were noted between the total amount of iron absorbed from the salt or complex under any of the conditions studied, suggesting that the mechanism of regulation of the absorption process at the mucosal level is the same for both drugs [13].

It was also established that the iron-polymaltose complex and iron sulfate are equally available for the hemoglobin recovery process. The 12-week study demonstrated by evaluating serum ferritin levels that the recovery of this metal in the body was more effective with ferrous sulfate.

Effects in iron deficiency anemia

Experiments in rats demonstrated that the morbidity of animals was associated more with the level of iron deficiency in the body than with a slowdown in hemoglobin synthesis. Pharmacological tests were developed to demonstrate the effect of Fe^{+++} complex in animals with experimentally induced iron deficiency anemia. The following three paragraphs provide an overview of these tests.

Rats

Experiments on the treadmill were carried out in healthy rats and animals with iron deficiency. Anemia was compensated as a variable by optimizing the hemoglobin level of all animals to the same concentration. At a level of hemoglobin necessary for carrying an adequate load, a significant impairment of the ability to run was observed in iron deficient animals in comparison with the control.

Iron therapy helped to restore the deficiency in 4 days. The cytochrome and myoglobin pigments concentration, as well as the activity of the processes of oxidative phosphorylation of substrates such as pyruvate malate, succinate and alpha-glycerophosphate in mitochondria preparations from skeletal muscles in rats with iron deficiency were reduced. A significant increase in the activity of only alpha-glycerophosphate phosphorylation alongside with productivity recovery occurred in iron-deficient rats receiving iron dextran.

Another group of authors from UCLA studied the effect of moderate to severe anemia on biochemical tissue parameters in adult male rats over time. Having formed 9 groups of Sprague-Dawley rats, the authors held 8 of them in a state of moderate anemia (8 g of hemoglobin/dL) for different periods of time from 3 to 360 days, and one group in severe anemia (4 g hemoglobin/dL)

for 30 days. The plasma iron concentration was reduced in the groups of moderate and severe anemia throughout the experiment in comparison with healthy control animals. As a result, the following conclusions were made: 1) moderate iron deficiency anemia does not cause a change in oxidative and glycolytic activity in skeletal muscles, whereas in severe iron deficiency anemia a rapid change in the mitochondrial component of skeletal muscles is observed; 2) blood analysis and biochemical study of muscles in the group of moderate anemia suggest the manifestation of hypochromic, microcytic iron-deficient anemia, while in the severe anemia group hypochromic, microcytic symptomatic iron deficiency anemia is observed.

The state of iron deficiency in young rats was obtained by feeding the animals with low-iron food for 8 weeks. The lack of iron led to a 50% decrease in the activity of cytochrome C, cytochrome oxidase and a 26% decrease in mitochondrial glycerol-3-phosphate dehydrogenase activity in skeletal muscles. The respiratory capacity of the muscle homogenates was reduced by 55%. After 8 days of iron administration, the respiratory capacity, activity of cytochrome C, cytochrome oxidase, and glycerol-3-phosphate dehydrogenase were approximately 50% closer to normal. The maximum level of oxygen consumption with a limb muscle contraction, on average, was 8 μmol O_2 / (min * g) in the control; 4.3 μmol O_2 / (min x g) in the iron deficiency group and 6.2 μmol O_2 / (min * g) in the 8-day iron balance recovery group. The level of muscle fatigue after 10 minutes of stimulation was higher in the iron-deficient group. Lactate concentration in the red musculature after stimulation was also higher in iron-deficient rats compared to the control. The levels of muscular fatigue and lactate response by 50% returned to normal after 8 days of iron therapy. The investigators concluded that the lack of iron in the body leads to a decrease in the activity of aerobic metabolism in the skeletal muscles, resulting in increased susceptibility of the muscle to fatigue [17].

Sheep

A rapid increase in the mass of red blood cells was noted with intra-amniotic administration of iron to the progeny of anemic sheep. This study tested the hypothesis that with the daily intra-amniotic administration of iron preparations to non-anemic sheep, in conjunction with intravascular injections of erythropoietin, the production of erythrocytes is enhanced. In the studies nine sheep fetuses were administered 100–120 units of erythropoietin intravascularly daily in the late gestation period in combination with intramuscular injections of 10 mg of iron for 7 days (erythropoietin low-dose group). Four more fetuses received daily 1000 units of erythropoietin and 10 mg of iron for the same period of time (erythropoietin high-dose group). The answers were compared with the indices of 9 control fetuses not receiving drugs. The results showed that immediately after the administration of erythropoietin, its concentration increased 25 or 250 times in the low and high dose group, respectively. The erythropoietin concentration returned to the baseline level 24 hours after the injection. The plasma iron concentration increased in the erythropoietin low-dose group, but not in the control or in the erythropoietin high-dose group. The reticulocyte index increased in both groups receiving erythropoietin, but not in the control. The hematocrit level increased in comparison with the control by day 5 in the erythropoietin low-dose group and by day 2 in the erythropoietin high-dose group. The gas content in the fetal blood did not change in any of the 3 groups throughout the experiment. From the above data, it can be concluded that, although the daily combined administration of erythropoietin and iron to non-anemic sheep leads to a significant dose-dependent increase in the mass of circulating red blood cells, this increase has only a slight similarity with the rapid increase in red cell mass following the administration of iron to fetuses with anemia caused by bleeding [18].

Iron is a natural component of the human body, which makes it one of the vital substances. It is a component of hemoglobin, myoglobin and a number of enzymes. Iron of hemoglobin is used to transfer oxygen. It is required for the metabolism of catecholamines and the adequate activity of neutrophils. The body contains about 4 g of iron, most of which is contained in hemoglobin. A third part of the total iron in the body is stored in the form of ferritin and hemosiderin.

The use of iron preparations makes it possible to correct the disturbances of erythropoietic disorders associated with iron deficiency. Iron can be in tri- or divalent form. Unlike Fe (II), Fe (III) ions form a high-molecular-weight polynuclear iron hydroxide in a weakly acidic medium (pH about 2). The iron (III) hydroxide complex with polymaltose is an organic complex with trivalent Fe (III), which is an anti-anemic nutritional supplement.
Experiments showed that there is no difference between the therapeutic activity of iron (II) and iron (III), as well as there is no difference in the bioavailability of iron from salts or complex.

2.2.2.2 Secondary pharmacodynamics

In vivo studies
Effect on the metabolism of Ca, P, and Mg

Rats

A group of Spanish authors studied the effect of iron deficiency on the utilization of Fe, Ca, P and Mg in rats. In addition to the well-known depletion of iron in the liver, femurs and sternum at low hemoglobin concentrations, iron deficiency in varying degrees disrupted the metabolism of Ca, P, and Mg. The change in status was not completely restored after the administration of iron, since minor signs of anemia in animals persisted by the end of the study. The second purpose of the tests was to evaluate the ability of the three iron compounds (citrate, ferrous sulfate and ascorbate) to correct various adverse effects of iron deficiency. After 10 days of treatment with these additives, magnesium absorption in iron-deficient rats was still reduced, especially in animals treated with iron citrate. Concentrations of hemoglobin reached normal values in all groups; however, the serum iron concentration remained low, indicating that iron stores in the body were still depleted. Concentrations of iron in the liver and femurs were also low in all groups, regardless of the type of additive obtained, compared with the corresponding controls; while iron concentrations in the sternum were significantly increased in all three groups, which indicated the activation of erythropoiesis. Concentrations of Ca, P and Mg in the liver reached normal values and normalized in the femurs. Only the content of Ca and P remained low when using citrate iron. In the sternum, known for high requirements in minerals, the concentration of Ca, P and Mg also increased. The obtained data showed that iron is involved in the processes of bone mineralization and, from the physiological point of view, has a beneficial effect on the metabolism of Ca, P and Mg, since its deficiency alters the status of these metals. These data also suggest that ferrous ascorbate and ferrous sulphate are absorbed more efficiently in the body than iron citrate [19].
This study demonstrates that iron deficiency in the body in varying degrees disrupts the metabolism of Ca, P and Mg. Iron insufficiency led to a disruption of magnesium absorption, a decrease in the calcium concentration in the liver, femurs and sternum, an increase in the content of P and Mg in the liver and a decrease in the P concentration in the femurs.

2.2.2.3 Safety pharmacology

Despite the fact that the IPC has a wide range of safety, since its acute toxicity is extremely low, and the side effects are few, some safety aspects of the drug should be noted. It should also be emphasized that, as described below in toxicity studies, the toxic properties of the drug depend on its dose, and the occurrence of adverse effects usually also depends on the administered dose of elemental iron.
Irritation of the gastrointestinal tract and abdominal pain, accompanied by nausea and vomiting, after oral intake are associated with astringent action of iron. Other gastrointestinal complications include diarrhea and constipation.
In case of iron overdose, in addition to gastrointestinal complications, such signs of toxicity as cardiovascular disorders including hypotension and tachycardia, metabolic changes associated with acidosis and hyperglycemia, central nervous system (CNS) suppression, which may vary

from lethargy to coma, hepatic insufficiency, blood clotting disorders, renal failure, pulmonary edema and death may occur [20].

Based on the observations made, it can be concluded that outside the overdose and subject to the administration of the proposed therapeutic doses of the drug, the development of adverse pharmacological effects or interaction with traditionally prescribed medications seems unlikely.

2.2.2.4 Pharmacodynamic drug interactions

Since iron preparations have been used in medicine for a long time in various dosage forms, such as tablets, syrups, drops or solutions for intravenous administration, now there are a lot of clinical and nonclinical data on its interactions with other drugs. Most of these interactions are insignificant and are mainly of scientific interest. But some interactions lead or can lead to serious consequences. Further an overview of the most significant interactions is provided.

Levodopa

In the last decade of the last century, more and more scientists have become adherent to the theory that iron accumulation and increasing levels of lipid peroxides in black substance are the main factors in the pathogenesis of Parkinson's disease. Even L-DOPA, which is widely used for the treatment of parkinsonism, undergoes autoxidation (like dopamine), resulting in the generation of reactive oxygen molecules. In one study of the Lee group from the clinic in Mount Siani in New York, the processes of lipid peroxidation in brain homogenates of mice were investigated. At the same time, the effect of iron (5 μmol ADP-iron), L-Dopa, dopamine, and ascorbic acid was investigated both in mono-administration and in the administration of mixtures. Activation of lipid peroxidation in brain homogenates took place under the influence of ascorbic acid or iron or both, which corresponded to other reports. The effect of L-DOPA was complex: L-DOPA powerfully suppressed lipid peroxidation, both in the presence and in the absence of ADP-iron. However, when L-DOPA was added in combination with ascorbic acid, no peroxidation suppression was observed, but an obvious peroxidation stimulation was observed after the addition of L-DOPA and ascorbic acid and iron. Deferoxamine, a potent iron chelator, largely suppressed lipid peroxidation under any conditions [21].

Ciprofloxacin and ofloxacin

The purpose of the recent study by Rodriguez et al. was to evaluate the effect of aluminum and iron on the dissolution kinetics of ciprofloxacin and ofloxacin *in vitro* and the importance of such *in vitro* data to predict changes in *in vivo* absorption processes under the influence of factors such as those often recorded *in vivo* interactions between quinolones and cations. The results revealed significant changes in the dissolution profiles of these quinolones in the presence of cations, in particular Fe^{++}, which reduced the maximum amount of dissolved ciprofloxacin and ofloxacin by 34.7% and 29.1%, respectively. Al^{3+} also caused a decrease in the total amount of dissolved quinolone, but it was less evident than Fe^{2+} [22].

Tetracyclines

A colorimetric test with siderophores found that three drugs of the tetracycline series (tetracycline, doxycycline and minocycline) are iron chelators. Determination of the minimum inhibitory concentration (MIC) showed that doxycycline activity against the periodontal pathogen *Actinobacillus actinomycetemcomitans* only slightly changed in the presence of excess iron, which apparently indicated the saturation of the antibiotic. On the other hand, the MIC of doxycycline and minocycline against *A. actinomycetemcomitans* was significantly reduced when microorganisms were cultured under conditions of iron deficiency compared to high-content conditions [23].

Penicillamine

The literature data indicate that the trifunctional amino acid D-penicillamine (D-P) causes various muscle disorders, the mechanism of which is unknown. The hypothesis of a group of British researchers was that such disturbances can also be a consequence of the influence of D-P on the

activity of protein synthesis, possibly by changing the metal content in the muscle. Male Wistar rats were injected DP intraperitoneally at doses of 50 and 500 mg/kg body weight. The rats of the control group received 0.15 mol/L of NaCl. Twenty-four hours after the administration a decrease in protein content in muscle and protein synthesis capacity was noted in skeletal muscles of rats treated with D-P (ratio of RNA: protein) fractional protein synthesis levels, synthesis rate per RNA unit and synthesis rate per DNA unit. Changes in the metal content included a decrease in the concentration of copper, iron and manganese with unchanged concentrations of zinc and magnesium. In the liver, D-P reduced the content of copper and iron, whereas the concentration of zinc, magnesium and manganese did not change. These effects of D-P could be direct, as the plasma indices of the damage degree of liver (activity of alkaline phosphatase and alanine aminotransferase) and kidneys (urea, creatinine and electrolytes content) did not change significantly under the influence of D-P [24].
The described studies indicate that iron can stimulate lipid peroxidation in the brain, reduce the quinolones content and increase the tetracycline MIC. Penicillamine reduces the iron concentration in muscles and liver.

2.2.3 Pharmacokinetics

2.2.3.1 Analitycal methods

Not applicable.

2.2.3.2 Absorption

On the basis of available literature data, it can be concluded that until now no differences in the mechanism of iron absorption in humans and other mammals have been identified. In all studied species of animals, the pharmacokinetic features of saturation of the transport system of water-soluble iron (II) and (III) preparations were significantly similar. Thus, it can be concluded that both rats and people without anemia can be used to assess the mechanisms of iron utilization or absorption [8]
In vitro studies
The process of iron absorption consists of two stages. The first step is the transport of iron through the villous membranes of the upper sections of the small intestine using specific transporters with biologically regulated activity. The second step is the interaction of iron with binding sites on the inner side of the membrane. The activity of their constituents is regulated by the concentration of iron itself. When more iron is ingested, absorption can also occur by diffusion, that is, by "penetration through the mucous membrane" [25].
Rats were shown to have an anatomical gradient of iron transfer. It varies from 27.40 ± 3.29% in the duodenum to 0.47 ± 0.19% in the ileum. The corresponding figures in animals whose iron content was insufficient or excessive were 45.30 ± 13.19% and 13.20 ± 0.89%, respectively, in the upper parts of the small intestine. In addition, with the use of bound small intestine loops *in vitro*, it was demonstrated that the processes of iron absorption in the mucosa, absorption and accumulation of intracellular binding proteins are regulated by cytoskeletal inhibitors [14]
The effect of iron (III) maltol on lipid peroxidation was compared with that characteristic of iron (II) sulphate, lecithin lipisomes, intestinal villi and mitochondrial preparations of the small intestine of rats. At a concentration of 100 μmol and higher, iron (III) maltols activated lipid peroxidation to a lesser extent than iron (II) sulphate. Maltol by itself can slow the transition of Fe^{3+} to Fe^{2+}. Thus, maltol can be a less toxic alternative to iron (II) salt for oral treatment of iron deficiency [26].
The level of radioactive iron [^{59}Fe] accumulation in isolated fragments of the small intestine of rats in the presence of two hydroxypyrones, maltol and ethylmaltol was compared with that in the presence of another iron (III) chelator, nitrilotriacetic acid (NTA). Absorption rates were similar

for all three ligands. Saturable absorption kinetics was observed between the iron concentrations of 10(-6) M and 10(-4) M. The capture of iron was almost inhibited by metabolic inhibitors. In the presence of pyrones at an iron concentration of 10(-4) M and higher, the absorption kinetics did not change by metabolic inhibitors and remained unsaturable. At low iron concentrations (10(-6) M), 35–40% of the absorbed metal was in a bound state with proteins, the molecular weight of which was similar to ferritin and transferrin. At high concentrations (10(-3) M), most [^{59}Fe] were in the fraction with a low molecular weight. The transition of iron (III) to iron (II) can occur on the surface of the mucous membrane cells before absorption by the saturable system. Maltol, ethylmaltol and NTK can retain iron (III) in solution and transfer it to the endogenous capture system. However, hydroxypyrones may be more suitable ligands for orally administered iron compounds, since in combination with the latter they do not exert a toxic effect associated with the combination of iron (III) and NTA and iron (II) preparations. The study results confirm that the iron polymaltose complex is well absorbed and utilized in rats following oral administration. The level of absorption increases in anemic animals and is identical with that of ferrous preparations. Bioavailability of the drug in the experiments was the same as that of other drugs. Containing bivalent and trivalent iron [27].

In vivo studies

Rats and human

Both experimental animals and human had equal ferrum absorption rate in the wide dose range for ferrous salts and iron polymaltose complex (III) under normal conditions. In 5 μg dose, absorption level of ferrous sulphate (II) and iron polymaltose complex (III) was: 1.8 ± 0,17 μg and 1.9 ± 0.11 μg (37.6% and 39.8%), and in 25 μg dose it was: 9.9 ± 1.66 μg and 9.6 ± 1.25 μg (39.7% and 38.4%), respectively. With the dose increased to 50 μg, absolute values of the absorbed ferrous salts and complex grew to 20.2 ± 3.29 μg and 12.0 ± 1.79 μg (40.4% and 24.0%) ($0.001 > p > 0.005$), and with the dose increased to 250 μg, the average level of absorption was 96 ± 9.15 μg and 47 ± 5.30 μg (38.4 and 18.8) ($0.005 > p > 0.001$). In animals after venesection following oral administration of 50 μg dose the values were as follows: 34.5 ± 1.67 μg and 25.1 ± 1.47 μg (for the sulphate and iron complex) (69.1 and 50.1%); and in case of 250 μg dose administration – 103 ±14.09 μg and 89 ± 11.65 μg (41.2 and 35.6%) ($0.7 < p < 0.06$). The level of hemoglobin contents were comparable. Increase of ferrum absorption activity after venesection can be only associated with the decrease of its content in spleen [14].

In animal investigations, relative bioavailability of 5 and 25 μg doses was comparable, but with the dose increase till 50 or 250 μg of ferrum, statistically significant prevalence of the salt compared with the complex was observed, which may have reflected absorption routes of these two drugs. The difference disappeared in iron-deficient rats after phlebotomy: in 250 μg dose, iron from polymaltose complex was as available for hemoglobin synthesis as from sulphate [14,28].

Rats

Evaluation of pharmakokinetics of ferrous sulphate and iron (III) hydroxide polymaltosate complex in rats with and without anemia found discrepancies in serum iron content after absorption, invasion and excretion constants, and volume of distribution of the two drugs. Nevertheless, the level of iron absorption and utilization in both drugs was similar. It is obvious that ferrous sulphate (II) and iron (III) complex use different absorption and/or distribution mechanisms [29].

Absorption takes place mostly in duodenum and in upper jejunum and progressively weakens in distal segments of intestine, [14]but its precise mechanism is not known. Ferritin and transferrin can contribute to iron absorption, but there are data on existence of specific iron receptors on the surface of villous membrane of gastrointestinal tract, which control iron absorption. These receptors and other unknow iron absorption mechanisms may possibly differ between the genders before pubescence [30].

In animals with iron deficiency anemia, a trend for absorption of higher amount of iron is observed. This figure is approximately proportional to the level of iron deficiency. In animals without iron deficiency, lower percent of this metal is absorbed. Equally significant discrepancies were detected in respect of supplements containing $FeSO_4$.

A group of scientists from University of California, Berkeley studied in detail the patterns of stimulating doses recommended by World Health Organization, supplements containing $FeSO_4$in Sprague Dawley male rats with iron deficiency and normal iron content. During 12 days after weaning, animals were treated less than 20 (iron-deficient group) or 400 microgram (normal iron content group) of Fe twice a day before feeding, followed by feeding with Fe-free feed AIN-76 during 1 hour. After that, each group (both iron-deficient and normal) was divided into three more groups, one of them continued initial diet, two other groups received 4000 microgram of Fe each before meals on a daily basis or every 3 days, i.e. according to frequency of regeneration of gut lining. During the experiment, evaluation of food consumption, hemoglobin concentration and the level of absorption and losses of ^{59}Fe was carried out. Anemia, low appetite and growth impairment were observed in rats with iron deficiency. The level of iron absorption was sustainable in the normal group (34.2%), the group of deficiency (89.7%) and the normal group regularly receiving a supplement (9.5%). Absorption activity reduced logarithmically in rats receiving iron on a daily basis, whereas in animals with iron deficiency receiving iron alternatively, absorption level decreased slowly and linearly. The degree of iron loss was high in rats receiving the metal on a daily basis. Total level of iron content during 13 days in normal and deficient rats receiving a supplement alternatively was 62% and 86%, respectively, of the level identified in animals receiving iron on a daily basis. Iron administration correlated with the time of gut lining regeneration was more effective [31].

Heme iron is absorbed actively. Non-heme iron is less available, and its reduced level of absorption decreases even more affected by intake of other meals.

Ascorbic acid in the form of a supplement or taken with food promotes recovery of iron iron to iron compounds (II), which activates absorption of non-heme iron [25].

Wistar male rats weighing 200–220 pounds were used in experiments. Iron was administered in two doses: 1.4 mg Fe/kg and 5 mg Fe/kg. Doses of non-organic iron (II) and (III) were administered in 1 ml of natural saline solution using a gastric tube. A lower dose of 1.4 mg/ kg can be compared with the standard iron dose (for an adult person weighing 70 kg this dose is approximately 100 mg or iron). A higher dose of 5 mg/kg corresponded to saturation area of dose-dependent absorption of glands after single dosing of the drug to rats. The level of iron accumulation in animals with the metal deficiency was predictably higher than in healthy ones. After administering a lower dose (1.4 mg/kg) to rats with iron deficiency, absorption of the drug was 5–14 times more than in normal animals. After administering a higher dose (5 mg/kg) absorption in rats with iron deficiency was 8–10 more than in normal animals [8].

Indication and distribution of [^{59}Fe]-ferro-^{3}N-maltol after intravenous administration was studied in anesthesized rats. Even after administration of high ferrum maltol doses (containing up to 1 mg of elemental iron), immediate dissociation of iron iron from maltol was observed in the blood flow. Excretion level of ^{59}Fe and tritium from plasma and their further destiny was different. The half life of ^{59}Fe from plasma was about 70 minutes, and its accumulation was mostly in the marrow and the liver. Tritium excretion from plasma was initially quick with the half life of 12 minutes. Entrance of 59Fe and tritium to blood plasma after the administration of [^{59}Fe]-ferro-3N-maltol to duodenum also differed. With low doses of ferrum maltol (100 μg of elemental iron), tritium appeared in plasma in maximum concentrations during several seconds, later on its content decreased against slow increase of concentration [^{59}Fe]. With higher doses of ferrum maltol (7 mg of elemental iron), levels of [^{59}Fe] content in the plasma reached maximum values during 5 minutes, then reduced against even increase of tritium concentration. [^{59}Fe] processing at the level of mucous membrane prevented high iron doses from entering to the circulatory bed [32].

Absorption of ^{59}Fe from ferrum maltol was studied in rats, which were orally pre-administered non-radioactive ferrum maltol containing 7 mg of elemental iron during two weeks. The level of ^{59}Fe accumulation in tissues during 2 hours after administration of radioactive ferrum maltol to the stomach was significantly reduced in animals receiving iron beforehand, compared with control group receiving natural saline solution. Activity of ^{59}Fe absorption from ferrum maltol in isolated ileum fragments and in duodenum was far lower compared with the absorption in small intestine. Profiles of plasma tests using gel chromatography in 5 and 60 minutes after intraduodenal administration of [^{59}Fe]-ferro-^{3}N-maltol demonstrated that the metal and ligand do not come into the circulatory bed in the form of complex even in case of increased permeability of intestinal wall [33].
Compounds that are natural and synthetic chelators of iron affect the absorption of the latter, and some of them can be used in treatment of diseases related to disorder of intestinal iron absorption [34].
Absorption takes place mostly in duodenum and in the proximal jejunum and progressively weakens in distal segments of intestine. However, its precise mechanisms are not known. Animals with iron deficiency tend to absorb higher amount of iron, approximately proportional to the degree of its deficiency in the body.

2.2.3.3 Distribution

Ferrum is distributed in the body bound with transferrin (glycoprotein beta-globuline) and is carried in the form of complexes with transferrin to the marrow, where it is included into hemoglobin during generation of red blood cells. Iron can circulate in blood only in trivalent form. Iron contained in iron (III) hydroxide polymaltosate complex does not bind to albumin or other plasma proteins. Small iron excess combines with apoferrin, as a result ferritin is generated, which is deposited in mucous cells. About 70% of iron is represented in the form of hemoglobin, 25% is contained in the form of ferritin and hemosiderin in iron deposit areas, 0.5% is contained in heme-containing enzymes and 0.1% is contained in transferrin [1].
Iron is a natural element playing vital role in many cell processes. The challenge is that iron in environment is permanently represented by non-solvable Fe^{3+}-form, which reduces its bioavailability for the body, as even small iron concentrations catalyze generation of damaging reactive oxygen molecules. As a result, in the course of evolution, live bodies developed the system of effective iron absorption and transfer designed to extract iron from the environment, as well as ferritins promoting iron accumulation and storage in a non-toxic form. In higher organisms, the first membrane barrier is apical surface of duodenum enterocytes, specialized absorbing intestinal epithelial cells providing vectorial transport of iron. Initially, iron is transferred into a soluble form by means of recovery, and Fe^{2+} ions are carried through cell membranes assisted by special mediators. Afterwards, iron is delivered inside the cell to basolateral membrane of enterocytes, comes through it and binds to blood transferrin in the portal vein system. The second sector of iron transport is placentary-fetal barrier transfer through which is performed using the same mechanism [35].
Three stages of iron absorption are known: 1) entrance to intestinal lumen and transport through apical membranes; 2) intracellular processing and transport to basolateral membranes; 3) exit of part of iron to the circulatory bed. Some newly identified molecules play vital role in this process. DcytB is a de-heme protein belonging to cytochromes b561family, which recovers trivalent ferrum ions in intestinal lumen prior to absorption. DMTI is an apical proton transporter of bivalent metals cations, providing iron transfer inside the cell. IREGI is a basolateral transporter with multiple binding sites, providing further iron transport. One more additional protein, hephaestin, is required for basolateral transport and iron binding to tranferrin in the portal vein system. Staying inside the cell, iron binds to ferritins, iron deposit protein [35]

Iron transport through placenta includes binding of transferrin-saturated iron to transferrin receptors on the mother side of placenta, followed by iron release to acid vesicles. Further iron transfer to fetus apparently involves DMTI, IREGI proteins and cuprum oxidase (hephaestin), as all of them are expressed in placenta [35].

In vivo studies

Mice

Iron concentration in mouse milk is about three times more than its content in blood serum. Despite obvious evidence of the presence of transferrin receptors in mammary gland of the rodent, precise mechanisms of iron entry to breast milk are not known. During lactation period, iron content in the milk of mice with iron concentration in serum varying from 8 to 66 μmol was linearly dependent on the relationship of iron and transferrin concentration in serum. Increase of iron content in milk by 340 μmol by means of transgenic lactoferrin administration to mammal glands of animals did not affect the nature of dependence between iron content in the milk and relationship of iron and transferrin concentration in serum. The ratio of equilibrium distribution of ^{125}I- between blood plasma and milk corresponded to 0.2, which demonstrated that transferrin entering the milk through transcytosis provided no more than 6% of iron containing in the milk. Fluorescent-labeled transferrin after incubation with the mammal gland *in situ* was located mostly near the basal surface of gland alveolar cells. These experiments proved that the initial and speed-limiting stage of iron transfer to milk is binding to basal transferrin receptor. Theoretical model of iron concentrations correlation in serum and milk suggests that affinity of apotransferrin to basal recirculation system can exceed the one observed in cells of many other species [36].

Rats

A group of researches from Greece developed an experiment involving assessment of iron content in liver, spleen and brain of control rats and rats receiving excessive amount of iron in order to analyze iron and transferrin (TF) distribution. Wistar rats received 3% carbonyl iron as a supplement during 3 months or during 4 months intraperitoneally or intravenously in the form of iron polymaltose complex (total dose was 300 or 350 mg/rat, respectively). Iron concentration was evaluated using atomic absorption spectrophotometry, and iron and TF distribution was evaluated using histochemical and immunohistochemical methods, respectively. The highest iron content was registered in the spleen, followed by liver and brain of control rats. In case of excessive iron intake, iron concentration increased more actively in liver rather than in spleen, its content in brain did not change significantly. In liver, iron distributed across Kupffer cell inside the lobes, and accumulated in hepatocytes peripherally. No difference between the number of stained cells or staining intensity in order to detect TF was identified in liver or central nervous system (CNS) of control rats and animals receiving excessive amount of iron; no TF was detected in spleen. Centrilobular location of TF in hepatocytes was reported. In brain, response to TF was positive in oligodendrocytes, in vessel walls, in epithelial cells of choroid plexus and in some neurons. Thus, experimental iron overdose in rats resulted in activation of iron uptake mostly by reticuloendothelial (RE) cells and hepatocytes, which demonstrates exceptional significance of hepatocytes in iron metabolism. Activity of iron uptake by brain cells did not change significantly, most likely due to brain protection against iron overdose. Excessive iron uptake did not affect the distribution and TF content in the liver and CNS, while iron and TF visualization sites did not coincide. This fact indicates that TF is not only iron transporter, but also can perform other functions.

Iron is distributed in the body along with transferrin and supplied to the marrow, where it is incorporated into hemoglobin. About 70% of iron is contained in the body in the form of hemoglobin, 25% is stored up in the form of ferritin and hemosiderin, 4% in the form of myoglobin, 0.5% in the form of heme-containing enzymes, and 0.1% in transferrin. Normally, maximum iron concentration is observed in spleen, followed by liver and brain. In case of overdose, the degree

of iron concentration increase becomes higher in liver than in spleen. Iron content in brain does not change significantly.

2.2.3.4 Metabolism

After absorption and distribution in the body, iron is not exposed to metabolism.

2.2.3.5 Excretion

In vivo studies

Rats

There is no physiological iron excretion system in the body, it can be accumulated in toxic amounts. Most part of iron released during hemoglobin destruction comes to repository or is re-used in the body. Excretion is carried out mostly during peeling, i.e. during the regeneration of skin, gastrointestinal tract mucous membrane, nails or hair. Only trace iron amounts are excreted with bile and sweat, excrements and urine. Iron losses increase sharply in case of hemorrhage.

After absorption in the body, iron is excreted only in minor amounts. Normally, excretion through kidney with urine is not significant. Daily loss of 2% of administered dose was reported in rats. In relation to total radioactivity of the whole animal losses were about 9%. Three days after the excretion of iron dose, the level of excretion of non-absorbed fraction of radioactive ^{59}Fe on the 6 day was considered as iron absorption activity parameter. No information was available regarding gastrointestinal tract, where absorption of radioactive ^{59}Fe was going on.

Utilization of the same labelled ^{59}Fe compounds 23 days after oral administration was analyzed by identification of blood radioactivity. The content of ^{59}Fe in liver reflects the distribution of this fraction in the repository of iron administered orally previously [8].

2.2.3.6 Pharmacokinetic drug interactions

Pharmacokinetic interactions of iron are mostly associated with the absorption, but can also take place at the level of distribution and excretion.

Heme iron is absorbed actively. Non-heme iron is less available, and low activity of its absorption decreases even more affected by food consumed.

Conditions of A and C vitamins deficiency can affect iron transport, its metabolism and storage in the body [38]. Ascorbic acid when taken in the form of supplements or with food, recovers salts of trivalent iron to bivalent form, which contributes to the absorption of non-heme iron [25].

Hypervitaminosis A reduces iron concentration in liver [39].

Interaction between absorption processes of different metals are known; cadmium inhibits the absorption of iron supplied with food in low and normal concentrations [40]; additional iron intake significantly blocks cobalt absorption [41]; zinc does not affect iron absorption, but iron reduces the activity of zinc absorption, if the total ion content of these metals in food (iron and zinc) reaches 1.36 mg [42].

Together with vitamins, iron affects bioavailability of fluorine [43].

Natural and synthetic iron chelators affect its absorption, and some of them can be helpful in treating diseases related to gastrointestinal iron absorption [34]. Simultaneous prescription of deferiprone, the first active oral iron chelator, reduces morbidity and mortality rate in case of acute iron overdose [44]. In one investigation [45], simultaneous administration of deferiprone and diazepam allowed to reach zero mortality level after acute iron intoxication in rats, while mono-administration of diazepam only reduced mortality rate from 60% to 16%.

Vitamin E and selenium being antioxidants also reduced iron concentration in mice hearts after its overdose [46]. Selenium also increased the activity of iron excretion with urine [47].

Simultaneous oral administration of iron caused significant changes in Pk/Pd of ciprofloxacin intravenous dose [48].

Tetracyclines are known for their high affinity to generation of chelate complexes with multivalent metal cations such as Fe^{+++}, Fe^{++}, i.e. tetracyclines absorption can reduce by 50–90% and more [49].

2.2.3.7 Other pharmacokinetics studies

Not applicable.

2.2.4 Toxicology

2.2.4.1 Single dose toxicity

Mice, IV administration

After IV administration, toxic effects in mice were studied by means of histological test of liver, kidney, adrenal, lungs and spleen tissues. It was found that toxic effects can be anticipated based on chemical properties. The authors divided trivalent iron compounds into two groups: iron hydroxide complexes and iron salts. Complexes (sucrose, dextran BP/USP and dextrin) demonstrated low toxicity level, as they have shape and structure similar to physiologic ferritin. Salts, on the other hand, actively interact with some substances of human and animal bodies, which is the reason for multiple adverse effects, with generation of free radicals being the most serious of them [50].

Rats, oral administration

Free iron in the circulatory channel is extremely toxic. Comparative studies of oral administration of bivalent iron salts and iron polymaltose complex in rats showed that LD_{100} for salt was 350 mg/kg, while neither diseases, no deaths of animals were reported after administering the complex at doses exceeding 1000 mg/kg [28].

During iron LD_{50} dosing to Wistar rats with food, its pharmacokinetics, concentration at baseline and maximum content in serum, as well as mortality rate between genders were compared. Prepubescent animals were represented by three-week rats, pubertal rats were represented by 6–8-week animals, adult rats were represented by 6–8-month animals. Prepubescent female rats died far more often than male ($p < 0,01$), pubertal female rats died significantly earlier than male rates ($p < 0,04$), which was also reported in adult animals ($p = 0,02$). Iron content in serum at baseline did not differ significantly in prepubescent female and male rats, but iron concentration at baseline in pubertal female rats significantly exceeded the one in male rats ($p = 0,06$). After iron dosing, concentration peak in serum of female rats was much higher ($p < 0,03$). Iron absorption mechanism is not known completely thus far, and it obviously differs between genders, which can be the reason for different metal content in blood serum of male and female rats and different mortality rates.

Rats of different age received 750 mg of elemental iron per kg of body weight, which corresponded to LD_{50} of elemental iron for these animals.

Statistically significant differences in mortality rate between genders were reported among rats. Among prepubescent animals (3-week-old), 16/20 (80%) female rats and 8/20 (40%) male rats ($p < 0,01$) died during 24 hours. Among pubertal rats (6–8 weeks old), 24/40 (60%) female rats and 14/30 (46,6%) male rats ($p = 0,03$) died within 24 hours after iron dosing, whereas female rats died significantly earlier that male rats. 19 female and 17 male rats died within 10 hours after iron dosing ($p = 0,04$).

Mortality rate in adult rats (6–8 months old) were the same as in pubertal rats. 19/20 female rats and 19/20 male rats died within 24 hours. However, female rats died earlier than male. 16 female rats died withing first 2 hours after iron dosing, while only 10 male rats died during the same period ($p = 0,02$). In all groups studied, death occurred during the first 24 hours. Death of animals of neither gender was reported in the control group.

No statistically significant differences were identified between genders in the iron concentrations at baseline. Mean values of ± CO iron content at baseline in female rats were 36±14 (19.4–63.2) μmol and in male rats 27.7 ±8.2 (19.4–44.2) μmol/l, respectively.
6–8-week-old rats initial ± CO iron content in female rats significantly exceeded the one in male rats: 47.8±10.8 (in the range of 40–55.6) μmol/l and 34.8±6.6 (in the range of 27.9–39.9) μmol/l (p = 0,006), respectively. Comparison of iron content at baseline between genders revealed statistically significant differences between female rats of different age groups. For adult female rats, iron concentrations at baseline were much higher than those in prepubescent animals (p = 0,049). This difference was borderly significant in adult and prepubescent male rats (p = 0,06). Iron pharmacokinetics after the prescription of 750 mg of elemental iron per kg of body weight also differed depending on the gender. Female rats had far more higher peak content of ± CO iron (427 ± 468,in the range of 106.1–1582 μmol/kg) than male rats (141.5 ± 188, in the range of 38.6–805.8 μmol/l; p = 0.003). Iron peak concentration in serum established 60 minutes after the dosing to the most of female rats, and as a rule 30 minutes after the dosing to male rats [51].
A group of clinicians from Canada carried out Deferiprone test [(1,2-dimethyl-3-hydroxypyrid-4-on) (L1)], the first active oral iron chelator, in order to use it in clinical trials for patients with chronic iron overdose. Its efficacy in the prevention of morbidity and mortality rate in case of acute iron intoxication was not studied. The investigation was aimed at the identification of deferiprone ability to reduce mortality rate in rats after oral administration of toxic iron doses. Rats received 612 mg/kg of elemental iron with food, which corresponded to LD_{58}. Concurrently, a group of animals received the same oral iron dose, followed by intraperitoneal administration of 400 mg/kg of deferiprone (initial dose), then by additional deferiprone dosing of 200 mg/kg, 100 mg/kg and 100 mg/kg with 1 hour interval. The results showed that simultaneous administration of iron and deferiprone reduced the mortality level from 58% (11/19) to 15% (3/20) (p = 0,013). Deferiprone prescription was associated with iron excretion with urine (which was not reported in iron mono-administration) and with the production of red iron complexes with deferiprone. During histologic test, less amount of iron was detected in liver and gastrointestinal tract. Consequently, simultaneous administration of deferiprone reduced morbidity and mortality rate associated with acute iron overdose [44].

Rats, IV administration

During intravenous administration of ferrous sulphate and iron polymaltose complex, LD_{100} for the sulphate was 25 mg/kg of body weight, and no morbidity of mortality rate was identified for polymaltose iron in doses exceeding 500 mg/kg [28].

2.2.4.2 Toxicity after multiple doses

Mice, intraperitoneal administration, 4 weeks

Investigation of chronic toxicity in mice was carried out in order to evaluate iron concentration in tissues and the level of free radicals production under chronic iron overdose. Compared with placebo-treated control group, mice dosed with iron had rough and discolored wool, and lethargy developed with the increase of total dose of chronic load. In placebo control group and among mice with the total chronic dose of 100 mg of iron dextran, mortality rate was not reported. Mortality rate was 20% and 60% in mice dosed chronically with 200 mg and 400 mg of iron, respectively.
Thus, this investigation showed that chronic iron administration resulted in highly significant, dose-dependant increase of iron concentration in tissues and systemic production of free radicals (p < 0,001) [52].

Rats, oral administration, 3 and 6 months

In another investigation, iron overdose in liver was used for the induction of its damage during long period in order to identify iron ability to induce oval cells. Rats received a diet with 2% of carbonyl iron during 3 of 6 months. Repositories of excessive iron appeared periportally in

hepatocytes and some Kupffer cells. Pericentrally, iron deposits were less pronounced. Also, small oval-like cells, morphologically and immunocytochemically similar to CDE-derived oval cells were detected and identified. Initially they appeared periportally, and later on in small ducts and lobes close to the central region. They showed positive staining for alpha-fetoprotein, pi-class glutathione S-transferase and embroyonic form of pyruvate kinase. These cells contained very small amount of iron, and they were classified as iron-free. The main difference of oval cells derived from CDE and from cells overloaded with iron was that the latter gave negative response during staining for transferrin. This investigation showed that cell changes taking place in iron-overloaded liver of rats, are similar to those observed in these animals under conditions of hepato-cancerogenic diet and in rats with chronic exposure to alcohol [53].

Gerbilles, subcutaneous administration, 7 weeks

Cardiotoxicity and hepatotoxicity coupled with hemochromatosis have never been reproduced in other species. A group of British researchers managed to obtain severe haemosiderosis strongly pronounced in liver and heart by means of subcutaneous administration of iron dextran to gerbilles on a weekly basis during 7 weeks. By studying gerbilles 1, 2 and 3 months following the last iron injection, they observed consistent development of hemochromatosis in heart and liver of animals treated with iron Hemochromatosis development in the form of cicatrix-shaped fibrosis became clear in all animals 1–3 months after iron dosing to gerbilles. Iron content in cardiomyocytes of gerbilles gradually increased from 1 to 3 month and led to the development of heart hemochromatosis 2–3 months after the last iron dextran injection. Repeated parenteral administration of iron dextran to gerbilles led to the development of heart and liver hemochromatosis, aspect of which is similar to the one specific for the terminal stage of disease in human. This pattern enabled detailed study of the mechanism of iron-induced damage of tissues by free radicals, which are considered to be the main reason for the development of such impairment. Also it can be useful during evaluation of iron chelators efficiency from the point of view of long-term prevention of unhealthy effect of excessive iron on heart and liver [54].

2.2.4.3 Genotoxicity

In vitro studies

Mutagenic activity of elemental and salt form of iron (Fe) including compounds used nowadays as supplements and for food fortification was estimated in mutagenicity tests in *Salmonella typhimurium* strains and mouse lymphoma cells *L5178Y*. Except for poor response obtained with ferrous iron fumarate, neither of compounds caused mutagenic response in experiments with salmonella. In test with mouse lymphoma, responses were associated with iron compounds and/or with the process of recovery of trivalent iron (Fe^{3+}) to bivalent (Fe^{2+}) iron. Responses of elemental iron forms were equivocal. Electrolytic iron with relatively large particles and irregular shape provided negative response. Smaller carbonyl iron forms which after 4 hours of incubation bound to cells and were absorbed by them, induced mutagenic response with and without S9. Mutation incidence increase was reported with iron iron chloride and phosphate ($FeCl_3$ и $FePO_4$) only in the presence of S9. For the compounds of bivalent iron, iron sulphate ($FeSO_4$) and fumarate ($FeC_4H_2O_4$), positive responses were reported in the absence of S9. Iron chelate, sodium Fe(III)EDTA gave positive response both with and without S9. For this compounds, minimum effective doses (MED) of mutagenicity induction were determined and relation of MED obtained was calculated. MED relation varied from 1 for FeSO4 to 30 for carbonyl iron, which corresponded to LD_{50} under oral administration, established in animal experiments [55].

Blue asbestos is known to cause cell damage leading to the development of asbestosis, bronchogenic carcinoma and mesothelioma in human. The mechanism of asbestos carcinogenic activity is not known. In association with asbestos, iron is presumed to act as a catalyst in generation of reactive types of oxygen, which can damage DNA causing mutations and cancer. In this experiment, asbestos ability to induce mutations in hgprt+ V79 cells of Chinese hamster or

transgenic hgprt-, gpt+ V79 cells (G12) was studied. Treatment of μg/cm^2 of blue asbestos during 24 hours caused double increase of mutation incidence in gpt locus of G12 cells, but not in hgprt locus of V79 cells. Mutation incidence of gpt locus in G12 cells grew with the increase of blue asbestos dose. Mutations induced by blue asbestos were considered to be the result of reactive oxygen production catalyzed by iron associated with fibers. This statement was based on the fact that during the treatment of G12-cells in iron-free medium containing fibers with active from oxidation-recovery point of view iron eliminated using desferrioxamine B, mutation incidence in gpt-locus did not exceed the level reported in non-treated control. Besides, cell treatment with soluble iron in the form of 1.5 M iron iron ammonium citrate caused the increase of the incidence of gpt-locus mutation approximately 1.5 times more against the level of untreated G12 cells. Whereas, mutation incidence affected by iron iron ammonium citrate did not grow in hgprt locus of V79 cells. The authors also studied the effect of nitrogen oxide on the mutagenicity of blue asbestos in G12 cells. When treating G12 cells with blue asbestos at a dose of 3 μg/cm2 in the presence of compound being the source of nitrogen oxide, i.e. 200 μmol of diethyl triamine/NO, mutation incidence grew till the level exceeding additivity threshold in relation to data obtained during mono-treatment with blue asbestos or diethyl triamine/NO. The results obtained suggest that the presence of iron and nitrogen oxide can lead to either formation of other reactive mutagenic compounds, like peroxynitrite, or, as is the case with nitrogen oxide, to inhibiting of DNA repair enzymes promoting increase of mutations amount [56].

Ceruleoplasmin (Cp) was found to promote oxidative damage to DNA *in vitro*, as evidenced by the formation of 8-hydroxy-2'-deoxyguanosine and strand breaks when incubated with a cysteine metal-catalyzed oxidation system (Cys-MCO) comprised of $Fe^{(3+)}$, $O_{(2)}$, and cysteine as an electron donor. The ability of Cp to induce oxidative DNA damage was blocked by hydroxyl-radicals arresters, such as sodium azide and mannitol, by metal chelators, like diethylenetriaminepentaacetic acid, spin trapping agent 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and by catalase. Caeruloplasmin also caused double increase of mutations in pUC18 lacZ gene in the presence of Cys-MCO, which was measured as the loss of ability for alpha-complementation. Cp incubation in the presence of Cys-MCO led to the increase of carbonyl group content and to significant change in ferroxidase activity, as well as proteolytic susceptibility of matter. Tests with deoxyribose and salicylate hydroxylation showed that generation of free hydroxyl-radicals took place in reaction of Cp with Cys-MCO. Herewith, the release of Cu atoms from Cp molecule was observed, and conformational changes of molecule were registered according to the change of fluorescence spectrum. Based on the results obtained, activating effect of Cp on the induction of DNA damage and Cys-MCO mutagenicity was interpreted as the result of active oxygen formation. This may be referred to free hydroxyl-radicals, which are produced in the reaction of free copper Cu^{2+} released from Cp molecules damaged during oxidizing, and from hydrogen peroxide molecules H_2O_2 produced by Cys-MCO. Release of cuprum atoms from Cp affected by oxidative stress may not only activate the processes of reactive oxygen production, but immediately cause cell damage [57].

In vivo studies

We didn't identify descriptions of mutagenic effects *in vivo* of iron or iron supplements as is. However, one investigation by American team of authors describes mutagenic effects of nickel and iron combination.

In this investigation, authors were primarily focused on nickel known as toxic, mutagenic and cancerogenic metal posing major threat to the body and environment. Despite the fact that some cell targets of nickel were identified, solid amount of data indicate that it can indirectly affect DNA, inducing formation of oxidized purines or pyrimidines contributing to the formation of promutagenic regions. In the reported investigation, kidney sarcoma induced by nickel sulphide (Ni_3S_2) or Ni_3S_2/Fe in F344 rats was studied for the presence of transforming mutations in K-ras-oncogene. In the course of selective oligonucleotide hybridization assay of K-ras oncogene

sequences amplificated using polymerase chain reaction, it was found that 1 of 12 basic tumors induced by Ni_3S_2, 7 of 9 basic tumors induced by Ni_3S_2/Fe contained exclusively GGT→GTT activating mutations in the 12 codon. Such mutations correlate with known ability of nickel, in the presence of oxidizing agent presented by iron in this case, to catalyze formation of 8-hydroxy-deoxyguanosine, which in its turn causes dATP misincorporation opposite oxidated guanine residue. Presence of GGT to GTT transversion was confirmed by direct sequencing of Polymerase chain reaction products. Absence of transforming mutations as per 13 or 59-61 codons was also discovered in the course of sequencing. Additionally, direct correlation between the reduction of latent period of tumor and presence of activating ras-mutations was reported. These results showed that Ni3S2 can cause transforming mutations in rat kidney, which is coherent with oxidizing properties of nickel. Whereas, iron aggravating the degree of oxidative damage to cells may increase the incidence of such transforming mutations [58].

2.2.4.4 Carcinogenicity

Long-term studies

Carcinogenicity study was carried out in mice species susceptible to carcinogenic substances. Iron polymaltose complex was administered i.m. at a total dose of 180 mg Fe^{+++}/kg compared with other organic iron complexes. Only female mice were included into test animal group, with their condition being monitored till death, with burnt out tumors, their occurrence rate and latent period in comparison with control being registered. Conclusion made on the basis of test results, when iron polymaltose complex route of administration provided its complete absorption, was that the drug under consideration did not have stimulating effect on the formation of spontaneous tumors. Investigation of 400 rats, in the course of which iron (III) polymaltose complex was also administered i.m. at a dose of 180 mg/kg, showed no carcinogenic properties in it [59].

2.2.4.5 Reproductive and developmental toxicity

Fertility and early embryogenesis

It is well known that the deficiency of micronutrients during early embryonic development can lead to structural problems and/or embryo death. Recently, the interest in the issue concerning the negative influence of slight excess of natural metals on the development of embryo has increased. A group of scientists from University of California, Davis suggested a hypothesis that, from the point of view of toxic effect, metals with similar physical and chemical properties use similar mechanisms for the impact on embryo prior to implantation. In this investigation, they carried out analysis of impact of four natural micronutrients (Cu, Mn, Fe, Zn) and 8 non-natural micronutrients (Cr, Hg, Pb, V, Al, Ag, Cd, As) on mice embryo development before implantation. Mice embryos at bicellular stage were cultivated during 72 hours in the medium containing different concentrations of metals (0.05–200 μmol). Differentiation and proliferation of embryo cells was evaluated based on the analysis of blastocyte formation and final amount of embryo cells. Both natural and non-natural metals were toxic for embryo cells in relatively low concentrations. However, contrary to all expectations, similar molar concentrations of redox active natural metals were less toxic than of inactive ones in redox reactions of non-natural metals. This data indicates that direct binding of metals to critical sites of membrane and/or with intracellular ligands, including proteins and nucleic acids can cause impairment of development or death before oxidative damage associated with metals [60].

Impact of exogenic iron and folate to reproductive function of pigs can be interpreted in two ways. Although impact of exogenic iron and folate on the secretion of relevant transport matricular proteins has not been previously reported. Twenty breeding pigs (5 animals per group) were infused with 1) saline, 2) alpha tocopherol, 3) alpha tocopherol and iron citrate, or 4) alpha tocopherol and tetrahydrofolate on the 11 and 14 days of pregnancy. Intravenous administration of iron citrate and tetrahydrofolate ($p < 0.05$) correspondingly increased the content of iron and

folate in plasma 6–8 hours after treatment. These manipulations didn't have impact on the content of uteroferrin or secreted folate-binding protein in uterus lavage on the 15 day of pregnancy. These data allows concluding that production of uteroferrin and folate-binding protein in uterus doesn't depend on iron and folate content in plasma, hence it may explain two-way impact of iron and folate on the reproduction activity of pigs [61].

Embryofetal development

To study the impact of iron deficiency and anemia on placenta of female parent, analysis of the composition and vascularization of placentary labyrinth in rats with limited iron intake was carried out. Rats from experimental group were placed under the conditions of limited iron intake with food 1 or 2 weeks prior to mating and received such diet during all gestation period. Placenta was studied on the 21 day of gestation. Tissue segments were stained with lectin for identification of foetal capillars and analyzed using stereological methods. Area density of capillars and total area of capillar surface significantly decreased in both experimental groups receiving limited amount of iron compared with the control. Total length of capillars was significantly reduced in the group of limited iron intake 1 week prior to mating, but not in the limitation group 2 weeks prior to mating in comparison with the control. The volume of endothelial cells was increased in both experimental groups. There were no significant deviations from the control in terms of foetal capillars, volume of mother blood regions or surface area of female parent contact with the embryo. The volume of labyrinth, labyrinth tissues and surface area of female parent contact with the embryo were increased in 2-week group compared with 1-week. Such changes in the degree of placenta vascularization can contribute to growth impairment of the embryo observed under iron deficiency [62].

Preliminary study was conducted in order to analyze significance of calcium or iron deficiency in the diet for the reinforcement of embryotoxic and teratogenic effect of lead administered orally to hamsters. Female animals in the experimental group received water containing 0.05% or 0.1% of lead acetate and food with insufficient iron or calcium content during several weeks prior to and/or during pregnancy. Frequency of embryofetal death and development of abnormal changes in embryo significantly increased in offspring of these animals who were born prematurely (on the 15th day of gestation), compared with offsping of control animals also receiving water with lead, but against adequate nutrition [63].

Pre and postnatal development (including mother's condition)

During the perinatal period the brain requires for precisely regulated iron transport system. Regulating iron transport proteins 1 and 2 (RITP) are cytosolic proteins that control stability of mRNA of two major cell iron transporters – transferrin receptor (TFR) and bivalent metal transporter-1 (DMT-1). In the experiment the RITP localization, changes in their perinatal expression, and their relationship with TFR and DMT-1 levels in rat brain between postnatal days (PND) 5 to 15 were studied. Fixed frozen cerebral tissue 12 micron samples were obtained from offspring of Sprague Dawley rats on PND 5, 10, and 15. The samples were visualized under a 20–1000-power optical microscope to detect diaminobenzidine reaction after incubation with specific primary antibodies to RITP-1, RITP-2, DMT-1, and TFR; and universal secondary biotinylated antibodies, and tertiary antibodies. The RITP expression was increased parallel with the transport protein expression over time. RITP-1, RITP-2, and DMT-1 were expressed partially in choroid plexus epithelial cells on PND 5 and 10, and fully on PND 15. RITP-1, RITP-2, and DMT-1 have been widely expressed by cerebral vessels and ependymal cells by the PND 5. Glial and neuronal expression of RITP-1, RITP-2, TFR, and DMT-1 in cerebral cortex, hippocampus, and striatum was increased over time, and these changes included the number of cells and expression intensity depending on brain region, cell type, and age. Such changes reported in the course of the RITP and iron transporters expression are indicative that various periods of susceptibility to iron deficiency or supplementation are attributable to various brain structures [64].

Studies in which the offspring (newborn animals) received doses and/or was further investigated.

2.2.4.6 Local tolerance

Not applicable.

2.2.4.7 Other toxicity studies

Not applicable.

2.2.5 Summary and Conclusions

Currently iron deficiency is one of the most significant nutrition concerns in the world.
Iron has numerous biological functions, and that establishes the significance of the problem related to its deficiency. This metal is well-known due to its role in oxygen transportation by the blood. Found in hemoglobin that is essential for effective erythropoiesis, iron provides oxygen capture by the lungs, its transport, and release in the body.
Use of oral iron supplements is indicate for prophylaxis and treatment of patients with iron deficiency anemia (hypochromic and microcytic) or blood loss.
Iron also serves as a cofactor of a number of metabolic enzymes (including cytochromes, mitochondrial and other oxidative enzymes). Taken orally with food or as a food additive, iron penetrates into mucosal cells and is bound to the protein transferrin. Iron protein binding results in hemosiderin or ferritin production. Reticuloendothelial system and hepatocytes are the major iron storages. Iron flows from transferrin to intracellular sites by means of specific transferrin receptors. Transferrin-iron complexes bind to receptors, and the emerging tertiary complex is absorbed by receptor-associated endocytosis. With the sufficient iron content is achieved, transferrin receptors synthesis is delayed, and ferritin protein production is increased. Absorption is believed to be regulated by hematopoietic transcription factor (NF-E2) which determines correlation between intestinal transport and erythropoiesis under common control of chromosome gene [6].
It is possible to make general conclusions on main processes involved in the iron absorption and distribution based on close interrelation between physiology and biochemistry of iron metabolism, especially under iron deficiency conditions, in warm-blooded animals, particularly in mammals.
In both experimental animals and humans, absorption of bivalent iron from ferrous salts and iron (III) from polymaltose complex was equivalent quantitatively but took place using different absorption and distribution mechanisms. From this point of view, the iron consumption in GI tract may be increased through the adherence to common recommendations on composition of meals. Pharmacokinetic characteristics of iron were highly similar in all species. From 20 to 30 % of iron coming from diet was absorbed in species with iron deficiency, and 3–10 % in the absence of such a deficiency.
Ferrous(III) hydroxide polymaltose complex had a very low acute toxicity. In mice and rats the LD_{50} value was approximately 7,000 μg Fe^{+++}/kg when administered intraperitoneally which represents about 2,500 times maximum daily human dose.
Maximum oral dose was 1.665 mg Fe^{+++}/kg (500 times the recommended human dose). Administered at this dose, the drug product did not result in death, gastrointestinal pathologies, or have any systemic exposure. Studies showed that the iron polymaltose complex has a wide safety profile. In the experimental conditions it was not teratogenic and had no adverse effect on fertility. Also it did not initiate any spontaneous tumors. The iron polymaltose complex did not show mutagenic effects.
IPC (tablets, syrup, drops, ampoules for IM and IV administration) is an antianemic drug containing the element necessary for the formation of hemoglobin. It has favorable

pharmacological and toxicological profiles when used to prevent and treat conditions caused by iron deficiency.

2.3 Summary of Clinical Study Results

Introduction

This clinical overview is made with reference to Ferrum Lek chewable tablets and Ferrum Lek syrup within the framework of submitting documents to update the internal marketing licence for the drug products by the applicant.

The objective of this report is to provide the accurate and relevant information of iron preparations presented in the form of tablets, chewable tablets, and syrup. In particular, a review of recently published literature will be performed to provide detailed presentation of new safety and efficacy information associated with the above mentioned drug products. Sections in italics will include the critical evaluation of the presented data. Various databases were searched for published literature to reveal complete and up-to-date information (preferentially, in Medline system). The Summary of Product Characteristics (SPC) included in this application was drawn up by the applicant. The document does not contain any new significant information to be included in the approved version of the SPC. All publications cited are listed in Section 15 – References.

2.3.1 Product Development Rationale

Each Ferrum Lek ® chewable tablet contains 100 mg of iron(III) hydroxide polymaltose complex (IPC). Each 1 mL of syrup contains 10 mg of iron(III) hydroxide polymaltose complex (IPC). Each 5 mL of syrup (1 measuring spoon) contain 50 mg of iron(III) hydroxide polymaltose complex (IPC).

Iron can be used in various forms of administration. Oral administration is the safest and cheapest one, and results in lower number of complications [65]. Oral ferrous fumarate, sulfate, gluconate, and polysaccharide iron complexes are commercially available, and prescribed for prevention or treatment of iron deficiency.

Ferrum Lek ® chewable tablets and syrup are clinically tested medical products aimed for the prevention and treatment of iron deficiency unless there is iron malabsorption in the gastrointestinal tract. The listed drug products contain the non-ionized iron that is well-tolerated and typically does not cause adverse events.

Chemically a polynuclear iron(III) hydroxide core is surrounded by non-covalently bound polymaltose molecules. The total molecular weight of a complex is approximately 50 kDa. Therefore, the diffusion of the complex through the mucous membrane occurs 40-times slower compared to iron(II) chloride hexahydrate complex. The complex is stable and does not release ionized iron under physiological conditions. Structures similar to the physiological protein ferritin are formed inside the polynuclear core. Due to this similarity only trivalent iron from the complex is extensively absorbed. Iron(III) hydroxide polymaltose complex does not have pro-oxidative properties typical for ferrous salts [66,67].

The polysaccharide iron complex is brown amorphous powder, very soluble in water and insoluble in alcohol.

The iron(III) hydroxide polymaltose complex is a water-soluble non-ionised macromolecular compound of polynuclear iron(III) hydroxide and partly hydrolysed dextrin (polymaltose) chemically described as: $[Fe(OH)_3\ (H_2O)]n * [C_6H_{10}O_5]a$. Structural formula of the molecule is currently unknown [68].

Iron deficiency anemia is the most common nutritional problem and clinical condition in the world and represents the most progressing iron deficiency. It is characterized by reduced number or complete lack of iron storage sites, low serum iron, decreased transferrin saturation levels, and low

hemoglobin concentration or hematocrit values. Iron deficiency occurs when the volume of iron absorbed from the gastrointestinal system is not enough to meet the body demand due to the low amount of the iron that comes from diet, increased need for this metal or chronic blood loss. Amount of iron ingested and available for the synthesis of hemoglobin largely depends on patient's tolerance to the drug and their adherence to the recommended dosage regimen. The adequate therapeutic margin of the IPC used for iron deficiency anemia has been proved during clinical practice.
The iron(III) hydroxide polymaltose complex is used to treat the iron deficiency both for during its latency and upon manifestation, as well as to prevent iron deficiency during pregnancy.
Efficacy in treating iron deficiency and its prevention during pregnancy was proved by controlled studies in adults and children. The volume of iron inclusion into body processes associated with trivalent iron has been demonstrated to be equal to that of ferrous sulphate, i.e. there was no evidence that iron (III) is less effective (slightly less prominent daily increase of hemoglobin concentration during first three weeks of therapy with iron(III) hydroxide polymaltose complex provided efficiency equal to that of the ferrous sulphate patients in 9 weeks of treatment) [69,70].
IPC has a favourable pharmacokinetic profile. The polymaltose complex does not release ionized iron during its pass through the stomach, i.e. on most cases it does not induce gastrointestinal conditions. Radioactive iron studies have shown that after taking iron(III) hydroxide polymaltose complex the serum iron concentration did not increase as prominently it does after ferrous sulphate exposure. Additionally IPC is safe in simultaneous administration of other drug products as it does not decrease the iron absorption activity and does not call for interruption of iron therapy [71].
The iron deficiency therapy with iron preparations should be continued for several weeks after normal values of hemoglobin concentration are achieved in order to replenish the iron stores.
Ferrum Lek ® Iron(III) hydroxide polymaltose complex is registered as a drug product in the form of chewable tablets and syrup in Slovenia since 1994 and 1995, respectively. Iron(III) hydroxide polymaltose complex was first synthesized by Vifor (International) Inc. Lek, Ljubljana manufactures Freem Lek medicinal products using its own manufacturing process.
Multiple post-marketing data of observational studies proves that iron(III) hydroxide polymaltose complex has a good risk/benefit ratio and is one of the most efficient iron preparations.
The data suggest that IPC is generally well tolerated. The most frequent adverse events were such gastrointestinal tract irritations as abdominal fullness, pressure in epigastric region, nausea, constipation, and diarrhea. These reactions are extremely rare, easily tolerated, and transient in nature.
Efficacy and favorable risk-benefit ratio of iron(III) hydroxide polymaltose complex treatment have been shown for 20 years of the preparation (chewable tables and syrup) use in clinical therapy. In this report the summary of the results obtained is presented.

2.3.2 Overview of Biopharmaceutics

Not applicable.

2.3.3 Overview of Clinical Pharmacology

2.3.3.1 Pharmacokinetics

2.3.3.1.1 Absorption

Iron is absorbed unevenly and not completely from GI tract; it is mostly absorbed through duodenal epithelial cells and partially from the upper small intestine (jejunum) [91].
Regular absorption of iron from the GI tract maintains functional amounts of the metal, and supports a trend of its storing. Drugs with enteric coating and some slow-release medications may transport iron outside the duodenum and the jejunum which slows down iron absorption [72].

Iron absorption is a complex process influenced by many factors including route of iron administration, dose, presence of iron storage, erythropoiesis activity, and diet. Bioavailability of oral iron varies from less than 1% to over 50%, and amount of the metal pool is the major factor that regulated iron absorption in GI tract [71,72].
About 5–13% of iron coming from diet is absorbed in healthy people, and about 10–30% in patients with iron deficiency. In adults, the extent of alimentary iron absorption is about 6% in males, and approximately 13% in non-pregnant women of childbearing potential. During pregnancy the activity of iron absorption in GI tract increases to compensate tissues growth and blood loss during labor. However, the precise extent of such intensification of the absorption is not known. The absorption rate in GI tract also increases in patients with iron deficiency. Up to 60% of therapeutic ferrous iron dose may be absorbed in subjects with iron deficiency; however, absorption of inorganic iron is reduced when prescribed together with various food and some drug substances. Inorganic iron is known to be absorbed almost twice more actively than the alimentary one. Although it is not known exactly what iron form is absorbed, ferrous sulphate absorption is considered more effective [72,73].
Bioavailability of iron in oral preparations is affected by composition of meals, and amount of the metal stored in the body depot. The less the metal stores are, the more active its absorption is.
The absorption of bivalent iron compounds is more effective compared to trivalent iron preparations [65]. Difference in the absorption rates is primarily that in fact the trivalent iron compounds are slightly soluble at pH > 3, whilst iron (II) preparations are highly soluble even at pH = 7 [66, 67]. Iron (III) and (III) ions form insoluble complexes with some food components and drugs that are poorly absorbed. Decreased gastric acid secretion makes iron (II) absorption less effective.
The precise iron absorption mechanism is unknown. However, it is considered to involve two mechanisms at the same time. Active transport by enzymes or transporters takes place mainly upon intake of normal iron concentrations coming from food. Passive transport starts mainly upon intake of iron doses exceeding those of normal diet [74].
The iron(III) hydroxide polymaltose complex does not release ionized iron during its pass through the stomach, i.e. in most cases it does not induce gastrointestinal conditions. Absorption of the iron(III) hydroxide polymaltose complex does not require acid environment, making it effective in the treatment of iron deficiency anemia in the patients who underwent gastrectomy.
The radioactive iron studies have shown that the iron serum concentrations increased rapidly (within 2–6 hours) after intake of ferrous sulfate. There was no significant increase in the iron content in serum after an iron(III) hydroxide polymaltose complex dose, but iron inclusion in the liver was also reported in 28 hours after its administration. However, the volume of iron inclusion into body processes was similar for both medicinal products [74,75]. High iron (II) serum concentrations after ferrous sulphate administration results in iron-albumin interaction. Thus, the ferrous sulphate accumulates in blood, and volume of its inclusion into body processes does not increase significantly. To achieve the required absorption activity the ferrous sulphate it should be used 2 hours before meals, whereas the iron(III) hydroxide polymaltose complex can be taken during or immediately after meals with equal efficacy [75].
Absorption of iron complexes with moderate stability, irrespective of metal valency, differs from that of stable ferrous salts. Such stable complex as IPC releases iron into the transport system actively and directly on mucous surface immediately after passing through the stomach. This process is not accompanied by oxidation – recovery reactions with aggressive radicals release, as transport proteins can accept only trivalent iron, in the form that is contained in IPC. There are no free iron (III) ions as iron of IPC flows directly to an absorption site where iron-binding proteins are arranged. Once iron need is met, transport into mucosal cells stops, and IPC is naturally excreted from the body.

IPC remains soluble at pH 1 to 14 as it has optimised complex stability. It will decompose to trace amounts of iron (II) ions during stomach transit, and in the duodenum become available for transport.
A need for replenishment of iron stores provides a number of benefits to IPC as it has better tolerability that those known for ferrous sulphate. Such iron may be captured by enterocytes using the same mechanism which is used for alimentary iron capture. A similar explanation could justify relatively low toxicity of the drug and absence of concerns for its overdose in patients who have a pathologic iron accumulation gene expressed.

2.3.3.1.2 Distribution

Bivalent iron crosses the cells of the GI tract mucous membrane immediately into the blood and binds to transferrin. It supplies iron to the marrow where it is included into hemoglobin.
If the iron stock sufficient to meet all body needs, most part of iron (70%) is functional. More than 80% of the functional iron is represented in the form of hemoglobin in erythrocytes, and the rest part of it is contained in myoglobin and intracellular respiratory enzymes (the enzymes contain less than 1% of all iron stores present in the body).
Ferritin that is a soluble protein complex is the primary storage of iron (about 70% in males, and 80% in females) in the body. A lower amount of this metal is deposited as hemosiderin that is an insoluble protein complex. Ferritin and hemosiderin are produced primarily by the liver, reticuloendothelial system (RES), bone marrow, spleen, and skeletal muscle. Small amounts of ferritin also circulate in plasma [72].
Iron can circulate in blood only in trivalent form. Iron contained in iron (III) hydroxide polymaltosate complex does not bind to albumin or other plasma proteins.
It binds to blood transferrin the same as iron released during erythrocyte destruction. The excessive iron is stocked up in the form of ferritin and hemosiderin in the RES and reused when necessary. The transferrin-bound iron can be extracted from the blood using standard laboratory methods. Its normal concentrations are highly variable. Morning values exceed evening concentrations. On average, about 35% of transferrin is normally saturated with iron. The remaining protein is presented in free form and may bind to additional amounts of iron [74].

2.3.3.1.3 Metabolism

Iron metabolism occurs in a basically closed system. Most part of iron released during hemoglobin destruction is converted and reused in the body.

2.3.3.1.4 Excretion

The human body excretes 0.5–2 mg of iron daily. Sexually mature females and women of childbearing potential lose 0.9–1.0 mg and 1.5–2.0 mg of iron per day, respectively. Iron is excreted primarily in feces and shedding cells of skin, gastrointestinal mucosa, nails, and hair; and only trace iron amounts are excreted with bile and sweat.
Iron losses increase sharply in case of hemorrhage.
Radioactive iron studies showed that in sexually mature males and females 8% of the absorbed iron is excreted within 1 year, while women of childbearing potential lose up to 20% of iron per year.

2.3.3.1.5 Pharmacokinetic interactions

As iron contained in iron(III) hydroxide polymaltose complex is completely bound, reducing the absorption level insoluble chelates resulting from reactions with food ingredients (such as phytin, oxalates, and tannin) and related medicinal substances (tetracyclines, antacids) are not formed which happens when ferrous salt preparations are administered.

2.3.3.2 Pharmacodynamics

Iron is contained in molecules of hemoglobin, myoglobin, and a number of such enzymes as cytochrome, oxidase. A third part of the total iron in the body is stored in the form of ferritin and hemosiderin. In adults, up to 2.5 g of iron can be contained in hemoglobin, 1.5 g in the pool, less than 0.4 g in myoglobin, and about 4 mg in transferrin, respectively [72].
The recommended daily dose of iron in diet for adults and children older than 12 years should be 12 mg, for women of non-childbearing potential 10 mg, for pregnant women 30 mg, for women of childbearing potential and breast-feeding women 15 mg, and 10 mg for children from 1 to 2 years old. However, it should be considered that the average level of iron absorption is 10%. Standard iron loss is approximately 0.9 mg in adult males and 0.8 mg in adult females, and this value is regulated by level of iron absorption from food. Normally the volume of iron absorbed and excreted daily should be similar [73].

2.3.3.2.1 Mechanism of action

Iron is present in every cell and has several essential functions. Ionized iron is a component of several enzymes that are necessary for energy transfer (cytochrome oxidase, xanthine oxidase, succinate dehydrogenase), as well as some compounds that transport and utilize oxygen (hemoglobin and myoglobin). Cytochromes transfer electrons inside the cells. Hemoglobin carries oxygen from lungs to tissues, myoglobin helps to use and store oxygen in muscles. Iron deficiency can affect the aforementioned functions, which results in diseases and death [72].
The range of iron deficiency manifestations varies from iron depletion, which doesn't cause any physiological changes, to anemia, which leads to dysfunction of some organ systems. Iron pool depletion is characterized by the decrease of iron stock in the body, but the share of functional iron (for example, within hemoglobin) may stay the same. If patients with iron deficiency have an increased need for iron, there is no pool in their body, which could provide iron. Iron deficiency is characterized by the depletion of iron stock due to erythropoeisis, which further slows iron transport. Absorption in GI tract is not enough to substitute depleted stock or to provide the body with necessary iron for growth and functioning. Decrease of iron supply causes production of imperfect functional compounds containing iron, including hemoglobin; red blood cells become microcytic and hypochromic [73].
Pharmacodynamic action means iron deficiency effect (effect on red blood cells, reticulocyte response, hemoglobin recovery, effect on iron pool).
Iron preparations are used for the prevention and treatment of iron deficiency, but iron doesn't correct hemoglobin structure distortion, caused by reasons other than iron deficiency. When such preparations are used to treat anemia, which is not related to iron deficiency, it can lead to the development of iron-storage disease.
Iron preparations compensate erythropoietic disorders caused by iron deficiency. Iron cannot stimulate erythropoeisis [72].
Iron preparations can also help relieve other symptoms of iron deficiency, such as tongue tenderness, dysphagia, skin and nails dystrophia, and cracking mouth corners [72].

Other effects

Iron is necessary for vital activity of such microorganisms as bacteria, because minerals are important both for the bacterial pathogenicity and their ability to support protective mechanisms of the host [72].
Due to several reasons, potential consequences of prescribing iron supplements to patients with iron deficiency have so far raised doubts. Some patients can be in a risk group for potential bacterial infection due to the use of such supplements (iron is also necessary for vital activity of microorganisms and can create favourable conditions for bacterial growth). Despite the fact that there is no evidence suggesting that small additional amounts of iron can make a healthy person more susceptible to infections, there are data demonstrating that in populations with high

prevalence of such endemic infections as malaria iron therapy can cause an increase in teh incidence of infection complications [76]. However, several studies failed to demonstrate this harmful influence [77].
Iron can be associated with the development of ischemic heart disease due to the modification of low-density lipoproteins, which leads to an increase in their atherogenic potential. There is preliminary data allowing to suggest that introduction of ferrous sulfate to the diet can increase susceptibility of plasma lipoproteins to oxidation; however, no such effect was found when using non-ionized iron contained in the polymaltose complex [78,79].

2.3.3.2.2 Pharmacodynamic interaction

So far no such interaction has been observed. As iron is bound into complexes, its interaction with food components (phytin, oxalates, tannin, etc.) and related medicinal substances (tetracyclines, antacids) is unlikely.
Iron(III)-hydroxide polymaltose complex is practically inert from the point of view of interaction with multiple potential food and drug products ligands. This is justified at least in relation to time interval necessary for the passage through GI tract; one way or another, substances should preserve their ability to react with carrier iron-binding proteins.
Iron(III)-hydroxide polymaltose complex is safe in coadministration with other drug products and it does not lead to lowered the iron absorption activity and interruption of iron therapy [71].
Iron(III)-hydroxide polymaltose complex neither exerts oxidative stress, nor interacts with food components. Moreover, it was shown that administering this complex with a meal helps to increase the volume of absorbed iron.

Laboratory Changes

Although it was demonstrated *in vitro* that ferrous sulfate provokes false-positive reaction in fecal occult blood test, such effects are not revealed in vivo in patients receiving oral iron therapy (it might be due to *in vivo* iron being excreted with stool in the form of non-reactive insoluble precipitates).
Results of fecal occult blood test (hemoglobin test) are not altered, which makes it possible to continue treatment without interruption at the time of the test.
IPC is effective in the treatment of iron deficiency anemia (IDA). It can be taken with meals, which promotes patient compliance. IPC is known to have a pathway into red blood cells closely resembling that of alimentary iron, i.e. it is expected that when the pool is overfilled, absorption will slow down due to the switching of physiological mechanisms. Therefore, an assumption can be made that this drug product may play a potentially significant role in long-term supplementary therapy.
Low incidence of several adverse effects is favourable for prescribing IPC to patients susceptible to these effects and not eligible for fast iron recovery.
It was demonstrated that IPC is a highly bioavailable drug, which is well tolerated for long periods of time and is associated with minimum adverse events. Such observations are tremendously important for the iron therapy in general and in patients in particular, especially in the pregnant.
Based on the compatibility evaluation, we may conclude that oral iron(III)-hydroxide polymaltose complex can be coadministered with various medications, which will not alter the absoption of iron or other substances.
Pharmacokinetic and pharmacodynamic data are properly described in the Summary of Product Characteristics (SPC) submitted by the applicant.

2.3.4 Review of efficacy

2.3.4.1 Treatment of anemia (associated with iron deficiency)

Dose Ranging Studies

Iron(III)-hydroxide polymaltose complex was tested in a group of 113 children aged 6 to 48 months with iron deficiency anemia. It was prescribed for the period of 90 days at a daily dose of 2.5 mg/kg body weight with a meal or in the fasting state. A significant increase in hemoglobin concentration, HTC, and RBC count was noted in both groups [70].
Replacement therapy of iron deficiency anemia was based on iron preparation at a daily dose 2.5 to 4 mg/kg body weight [73].

Controlled studies

Several clinical studies showed that iron(III)-hydroxide polymaltose complex is highly effective in treatment of patients with iron deficiency anemia. The aim of these studies was to demonstrate the efficacy and tolerance of iron(III)-hydroxide polymaltose complex or to compare it with other iron preparations.
Several most significant studies are described in Table **1**.

Table 1 Comparative studies of iron(III)-hydroxide polymaltose complex

Reference	Design	Criteria for evaluation	Number of patients, sex, age	Dose, mg/day	Observation period	Results
Yakobs P. et al., 17	P, c, r, Comparison to FeSul	Hb recovery, pool recovery (ferritin level), AE	159 blood donors: 137F, 22M 32 (17-64)	FeSul: 60 mg/BID IPC: 100 mg/QD IPC: 100 mg/BID	12 weeks	Recovery of Hb: FeSul = IPC (both doses) IS In 12 weeks, FeSul > IPC with recovered pool AE: FeSul > IPC Withdrawal: 20% FeSul (nausea and vomiting) 0% IPC
Langstaff RJ et al., 5	D, b, m, Comparison to FeSul	Recovery of Hb, AE	104: 90F, 14M	IPC: 2 x 100 mg/QD FeSul: 3 x 200 mg/QD	9 weeks	Recovery of Hb: FeSul > IPC in 3 weeks (p = 0.03) and in 6 weeks (p = 0.005) FeSul = IPC in 9 weeks AE: FeSul > IPC (p = 0.03)
Bogdanova OM et al., 19	R, c Comparison to ferrous salt	Recovery of Hb, serum ferritin level, AE	69 elderly patients	FeSul: 60 mg/BID IPC: 100 mg/QD		IPC equally effective, good tolerance AE: minimum
Yakobs P. et al., 17	O, r Comparison to FeSul	Recovery of Hb, serum ferritin level, transferrin saturation % AE	Blood donors	IPC 100 mg IPC 100 mg + 0.9 mol/l GPH IPC 100 mg + 1.8 mol/l GPH FeSul 100 mg	12 weeks	Recovery of Hb: IPC = IPC + GPH = FeSul; Serum ferritin: FeSul > IPC AE: FeSul > IPC

Notes. P = prospective; r = randomized; c = controlled; o = open-label; IPC = iron(III)-hydroxide polymaltose complex; FeSul = ferrous sulfate; AE = adverse events; IS = insignificant; Hb = hemoglobin; BID = bis in die; QD = quaque die; p = likelihood; d = double; b = blind; m = multicenter; GPH = glycerophosphate.

Table 2Non-comparative studies of iron(III)-hydroxide polymaltose complex

Reference	Design	Criteria for evaluation	Number of patients, sex, age	Dose, mg/day	Observation period	Results
Andrade J. et al., 6	D, b, m,	Recovery of Hb, serum ferritin level	113 children	IPC: Syrup, 50 mg	6 months to 4 years	IPC = IPC taken with/without a meal; AE: IS in both regimens

Notes. d = double; b = blind; m = multicenter; IPC = iron(III)-hydroxide polymaltose complex; IS = insignificant.

Placebo-controlled studies

All placebo-controlled studies (from Cochrane database) of ferrous sulfate used during pregnancy explicitly showed that pregnant women who took iron had substantial iron store in the body, increased hemoglobin level, and decreased incidence of iron deficiency anemia compared to women who received placebo both during pregnancy and after that. Furthermore, children born of mothers who received iron therapy demonstrated increased serum ferritin compared to children from the placebo control group. Women took high doses of iron-containing supplements, 100 to 200 mg a day [80].

In a placebo-controlled efficacy study, 48 men with decreased iron pool volume were prescribed iron-containing supplements in the form of ferrous sulfate (180 mg iron a day), iron(III)-hydroxide polymaltose complex (200 mg iron a day), or placebo, which helped to demonstrate more beneficial action of non-ionized iron(III)-hydroxide polymaltose complex compared to ferrous sulfate [79].

Studies with active control

A prospective controlled randomized study of 159 healthy blood donors who developed absolute iron deficiency anemia compared action of 120 mg of ferrous sulfate and 100, 200 mg of iron(III)-hydroxide polymaltose complex. Both iron forms were equally available for hemoglobin recovery processes, but after 12 weeks of study it was shown that ferrous sulfate was more effective in iron store recovery, which was evident from the results of serum ferritin test. Toxic effects were more prominent in ferrous sulfate group, 20% of participants refused to continue therapy because of nausea and vomiting. Iron(III)-hydroxide polymaltose complex was associated with fewer complications as well as the absence of withdrawals [81].

The efficacy of iron(III)-hydroxide polymaltose complex in 126 healthy patients was compared to that of ferrous sulfate. It was shown that in ferrous sulfate group the hemoglobin concentration increased in 3 and 6 weeks, and in 9 weeks of therapy both groups demonstrated an equal hemoglobin concentration [69].

In a double blind multicenter study of children aged 6 months to 4 years with iron deficiency anemia, iron(III)-hydroxide polymaltose complex was equally effective regardless of simultaneous food intake. As far as therapy tolerance is concerned, both groups again demonstrated the same results [70].

In a comparative randomized study of 69 elderly patients with GI disorders and iron deficiency anemia, the efficacy of iron(III)-hydroxide polymaltose complex was compared to the one of ferrous salt. The drugs were highly effective in improving the disease course and recovery processes in both groups; tolerance of iron(III)-hydroxide polymaltose complex was good, and adverse events were minimal [82].

The only open-label randomized study of blood donors with anemia demonstrated that in 12 weeks of observation, iron(III)-hydroxide polymaltose complex and ferrous sulfate provided equal amounts of iron to launch the processes of hemoglobin recovery. There was an additional

evaluation of glycerophosphate, which was added to iron(III)-hydroxide polymaltose complex to activate iron inclusion processes. Increased iron inclusion due to the addition of glycerophosphate to the compound was not proven. However, iron(III)-hydroxide polymaltose complex was associated with fewer adverse events [86].
A more profound assessment revealed the following advantages of iron(III)-hydroxide polymaltose complex compared to ferrous sulfate in anemia treatment: a significant increase in hemoglobin concentration was noted after three days of IPC use and continued afterwards; it is supposed that long-term IPC use, recommended by WHO, will not be associated with toxic effect of iron; IPC can be taken with a meal, which does not influence absorption and helps to minimize adverse effects of the therapy; as a rule, IPC is well-tolerated, cases of therapy discontinuation or refusal are rare [84].

2.3.4.2 Prevention of (latent) iron deficiency

During growth, pregnancy, and breast feeding body needs significantly more iron. As a result, iron deficiency values should be carefully monitored within these periods. More than a half of women of childbearing age demonstrate a decrease of body iron store. Iron deficiency, which can reach 1000 mg, can develop during the first pregnancy trimester as a result of foetus development, placenta growth, and blood volume increase. During two following pregnancy trimesters iron need can increase up to 6.3 mg a day. As a result, most women develop iron deficiency. To prevent iron deficiency, in 80-90% of cases it is recommended to take additionally 50-100 mg of iron a day [80, 85]. Iron-containing supplements used during pregnancy should be suitable for the prevention of iron deficiency development in women.
A double-blind placebo-controlled study of 81 teenage girls with non-anemic iron deficiency demonstrated the improvement of learning abilities and memory due to additional iron intake [86].
A long-term observational study was conducted with participation of children who had been examined and took iron due to its deficiency since infancy. It was shown that children with severe chronic iron deficiency in infancy preserved psychological and behavioral abnormalities compared to patients with more than 10 years of treatment [87].
Data of controlled studies of iron-containing drugs used for iron deficiency prevention are shown in Table **3**.

Table3 Controlled studies of preventive iron use

Reference	Design	Criteria for evaluation	Number of patients, sex, age	Dose, mg/day	Observation period	Results
Milman N et al., 16	P, C, c, db	Hb recovery, IPC advantages	1277	40-200 mg	9 weeks – 3 months	Recovery of Hb: IPC > placebo, Serum ferritin level: IPC > placebo

Notes. C = Cochrane, db = database; p = placebo; IDA = iron deficiency anemia; Hb = hemoglobin.

2.3.4.3 Other unregistered indications

A prospective randomized study of blood donors who did not pass the copper sulfate test as the criterion for the donation, showed that iron(III)-hydroxide polymaltose complex treatment was beneficial for positive balance preservation, which helped to decrease the quantity of ineligible donors due to the iron deficiency anemia development after phlebotomy [81].
In a randomized double-blind placebo-controlled study, 250 blood donors submitted 2 units of red blood cells. Additional use of ferrous sulfate (100 mg) helped to prevent iron store depletion in most donors [88].

2.3.5 Dosing

Dose and treatment duration depend on the level of iron deficiency.
In iron deficiency manifestation, the treatment takes 3 to 5 months until the recovery of normal hemoglobin concentration. After that the therapy should be continued for several more weeks to replenish body iron pool [72].
In latent iron deficiency, the treatment takes 1 to 2 months.
Daily dose can be divided into several parts or taken as a single dose.
IPC chewable tablets should be chewed or swallowed whole and taken with food or immediately after food intake.
IPC syrup can be mixed with fruit of vegetable juices or added to bottles with infant nutrition. It should be taken with a meal or immediately after it. A measuring spoon for precise dosing is provided. Slightly colored solution does not influence taste or efficacy of the syrup.
Daily IPC doses recommended for prevention or treatment of iron deficiency are described in Table **4**.

Table 4Daily IPC doses for prevention or treatment of iron deficiency

	Dosage form	Iron deficiency manifestations	Latent iron deficiency	Preventive therapy
Infants (under 1 year)	Syrup	2.5-5 ml (25-50 mg of iron)	--	--
Children (1-12 years)	Syrup	5-10 ml (50-100 mg of iron)	2.5-5 ml (25-50 mg of iron)	--
Children (>12 years)	Tablet	1-3 tablets	1 tablet	--
Adults and breastfeeding mothers	Syrup	10-30 ml (100-300 mg of iron)	5-10 ml (50-100 mg of iron)	
Pregnant women	Tablet	2-3 tablets	1 tablet	1 tablet
	Syrup	20-30 ml (200-300 mg of iron)	10 ml (100 mg of iron)	5-10 ml (50-100 mg of iron)

Notes. (–) Due to the low dose of iron recommended for these indications tablets and syrup are not traditionally used dosage forms.

Children under 12 years
In case of iron deficiency manifestation in infants under 1 year, treatment starts with 25 mg of iron (2.5 ml, ½ of measuring spoon) in the form of IPC syrup with gradual dose increase up to 50 mg of iron daily (5 ml, 1 measuring spoon) in the form of syrup. Due to the fact that only very low iron doses are indicated for treatment of latent iron deficiency, tablets and syrup are not used for such indications. In case of breastfed preterm or underweight infants (less than 2.5 kg), preventive daily dose 2-4 mg/kg of iron (but not more than 15 mg) is prescribed at 2 months of age, better at 1 month. Full-term infants who are not breastfed of partially breastfed should receive preventive daily dose 1 mg/kg of iron during the first year of life.
In the case of iron deficiency manifestation in children aged 1 to 12 years, daily dose should be 50-100 mg of iron (5-10 ml, 1-2 measuring spoons) in form of IPC syrup. In the case of latent iron deficiency, daily dose is 2.5-5 ml (25-50 mg of iron) of syrup. Due to the fact that only very low iron doses are indicated for preventive therapy, tablets and syrup are not used for such indications. In the case of a sudden early pubescence, the need for additional daily iron intake can increase up to 2-5 mg in boys and girls, respectively.
Children older than 12 years, adults and breastfeeding mothers

In the case of iron deficiency manifestation, standard daily dose varies from 100 to 300 mg a day (1-3 chewable IPC tablets or 10-30 ml of IPC syrup, 2-6 measuring spoons). In the case of latent iron deficiency, daily dose varies from 50 to 100 mg of iron (1 tablet or 5-10 ml of IPC syrup, 1-2 measuring spoons). Due to the fact that only very low iron doses are indicated for preventive therapy, tablets and syrup are not used for such indications. Standard preventive dose for adults is 60-120 mg of iron a day.

Pregnant women

To prevent iron deficiency development during pregnancy additional iron-containing supplements should be recommended and prescribed on general or selective basis. As far as nutrition is concerned, selective prevention is preferable; women at risk of iron deficiency development are detected by means of ferritin in blood test. This approach helps women with adequate iron store to avoid excessive therapy, which could be potentially dangerous for 12-13% of heterozygotes and 0.3-0.5% of homozygotes for HFL gene, which codes hereditary hemochromatosis [92]. Preventive use of iron-containing supplements should stat with a dose of 30 mg a day.

In the case of iron deficiency manifestation, daily dose from 200 to 300 mg a day is prescribed (2-3 chewable IPC tablets or 20-30 ml of IPC syrup, 4-6 measuring spoons) until the recovery of hemoglobin concentration. After that the therapy should continue at 100 mg of iron a day (1 chewable IPC tablet or 10 ml of IPC syrup, 2 measuring spoons) until the end of pregnancy to recover iron pool.

In the case of latent iron deficiency and its prevention, daily dose from 50 to 100 mg of iron is prescribed (1 chewable tablet or 5-10 ml of syrup, 1-2 measuring spoons).

As far as the increase of hemoglobin concentration is concerned, IPC is almost equally effective as ferrous sulfate. Treatment efficacy is also the same regardless of food intake. Moreover, simultaneous food intake decreases the amount of adverse effects, which provides adequate adherence to recommendations.

IPC is the most suitable form of therapy for patients who do not need a fast iron store recovery, as well as for patients susceptible to adverse GI reactions, such as pregnant women and children.

Indications included by the applicant into the SPC were proven in clinical studies and comply with the current level of knowledge and understanding of the problem.

2.3.6 Safety review

Replacement iron therapy based on IPC is usually well-tolerated, much better than other iron drugs used for the comparison. Apart from minimal adverse effects profile (minor and temporary) it was shown that refusal, interruption, and discontinuation of therapy with IPC are rarely noted compared to other iron drugs. There is also no interaction with other medications. There is almost no ground for concern about malabsorbtion due to food intake or other drug products. IPC has an extended safety profile with minimal risk of accidental overdose. Additional important advantage of proposed drug is the absence of teeth coloring when using liquid IPC forms due to their non-ionic nature [74, 90].

2.3.6.1 Adverse effects

Adverse effects of using oral iron drugs include nausea, vomiting, abdominal pain, diarrhea, and constipation. Severity and incidence of such adverse effects depend on the amount of available ionized iron. GI toxicity is considered to be the consequence of direct mucous cells involvement [91].

Adverse effects associated with IPC use are, as a rule, minor and temporary. When using chewable IPC tablets or syrup, such GI symptoms as abdominal fullness, discomfort in upper abdominal cavity, dizziness, constipation, and diarrhea are rare [84].

A double-blind comparative study of 104 patients demonstrated that in the IPC group there were less adverse events, and there was a significant difference between patients receiving IPC or ferrous sulfate, as far as incidence of indigestion, nausea, and vomiting was concerned. Other adverse effects with 10% incidence noted in both therapeutic groups included abdominal pain, constipation, headache, and change of stool color [69].
In a multicenter comparative study of 113 patients (children) aged 6 to 48 months with iron deficiency anemia who received 2.5mg/kg of IPC for 90 days with or without food, only 3 patients of 113 had an adverse effect associated with iron therapy [70].
Monitoring of toxic adverse events showed that ferrous sulfate can cause gastric mucosa erosion or ulceration. This occurrence was not detected when using iron(III)-hydroxide polymaltose complex [68].
A randomized controlled study comparing toxicity and adverse effects range related to oxidizing action of ferrous sulfate and IPC in children with iron deficiency anemia showed only insignificant difference, however, SOD and LDL values were significantly higher in the IPC group [92].
Data of a comparative study with participation of 25 children with IDA who received IPC or ferrous sulfate showed that group there is a suppression of copper and ceruloplasmin metabolism in the ferrous sulfate group, whereas the IPC use did not influence the normal activity range of these processes [93].
A randomized study comparing bioavailability of iron(III)-hydroxide polymaltose and ferrous sulfate in blood donors with iron deficiency revealed such adverse effects as nausea and vomiting, which were less common in the IPC group. The difference was even more significant due to the fact that no donors in the IPC group interrupted the treatment on the grounds of adverse effects development [81].
As far as IPC tolerance is concerned, the drug is tolerated for long periods of time and is associated with insignificant minor adverse effects. This observation is especially important for iron therapy in the case of certain patients, such as pregnant women, children, and blood donors.
Effects causing treatment refusal
None.
Shifting from oral iron drugs
If a patient cannot take drugs orally, iron should be administrated intramuscularly or intravenously.

2.3.6.2 Overdose

So far there have been no documented intoxication or excessive iron stock events in case of overdose, as iron of active substance iron(III)-hydroxide polymaltose complex is not present in the body in a free form and does not enter cells through passive diffusion [68].
Literature reflects specific issues of iron overdose in children. Most cases of death occur in childhood, especially in the age from 12 to 24 months. As little as 1-2 mg of iron can cause death, however, lethal cases are usually related to the intake of 2-10 mg. The incidence of iron intoxication is defined by its domestic availability, particularly if there are certain supplements left after pregnancy. Colorful sugar-containing coating of most commercially available tablets makes them look like candy. Therefore, all iron-containing drugs should be kept in child-proof bottles [94].

2.3.6.3 Local tolerance

Not applicable.

2.3.6.4 Contraindications/Precautions

Ferrum Lek chewable tablets or syrup are contraindicated to patients with hypersensitivity to any of the drug components.

The drug is contraindicated to patients with excessive iron content or with disorders of iron utilization processes (hemosiderosis, hemochromatosis, lead anemia, sideroachrestic anemia, thalassemia), as well as with anemia not associated with iron deficiency.
The drug should not be prescribed to patients receiving repeated blood transfusion.
IPC should not be prescribed simultaneously with parenteral iron.
Precautions should be taken in patients with phenylketonuria (Ferrum Lek tablets contain aspartame, a source of phenylalanine in an amount equivalent to 1.5 mg/tablet).
Caution should be exercised in patients with diabetes mellitus. Insignificant increase in iron store concentration is associated with a significant increase in glucose homeostasis parameters [95].
Iron preparations are not used in patients with anemia caused by chronic inflammation or malignant neoplastic diseases, as iron is pooled in reticuloendothelial cells. Use of such iron to synthesize hemoglobin starts only after the elimination of pathology [96].
Oral iron drugs can color feces dark, but this effect is clinically insignificant.
There is data indicating that additional iron received by children without iron deficiency causes growth impairment [97].
If oral use of iron drugs was not successful, a possible reason could include incorrect diagnosis, a lack of compliance, constant bleeding, underlying infection, malignant neoplasms, liver disorders, and iron malabsorption.

2.3.6.5 Usage during pregnancy and lactation

Animal studies of efeects on reproduction identified no risk for offsprings. Controlled studies of pregnant women after the first pregnancy trimester showed no adverse reactions in mothers or the newborns. There is also no data on risk associated with this therapy during the first pregnancy trimester, negative influence on foetus is improbable [80].
Breast milk contains iron bound to lactoferrin. Only a small amount of iron from IPC complex is contained in breast milk; the likelihood of adverse effects in infants is very low. Toxic, cancerogenic, and mutagenic action of iron was not proven neither by means of clinical, nor epidemiological methods [80].
IPC tablets or syrup can be taken during pregnancy only after consultation with a physician.

2.3.6.6 Paediatric use

Chewable tablets are not recommended for use in children under 3 years, as there's a risk of aspiration. Consequently, young children should receive IPC syrup.
In the case of children under 12 years, syrup is also a more preferable dosage form than chewable tablets, as it simplifies dose selection.

2.3.6.7 Geriatric use

In the case of elderly patients with IDA, iron(III)-hydroxide polymaltose complex demonstrated high efficiency and good tolerance with minor adverse effects [83].

2.3.6.8 Use in patients receiving dialysis

Multiple studies showed that oral iron therapy is inadequate when treating iron deficiency in patients on dialysis. In the case of such patients, intravenous iron administration is preferable [98].

2.3.6.9 Influence on the ability to drive and use machines

No influence.

2.3.7 Post-marketing data

Apart from the well-known benefits of iron(III)-hydroxide polymaltose complex, there have lately been revealed other therapeutic advantages of such preparations.

Iron(III)-hydroxide polymaltose complex is a drug with slow release which enables only small amounts of iron to contact duodenal mucosa at a given period of time, increasing absorption and GI tolerance of the drug. Therefore, smaller amounts of iron provide the same therapeutic effect (compared to other iron drugs), and at the same time patient is ready to adhere to therapy recommendations thanks to the decreased amount of adverse effects. The drug demonstrates desired safety profile compared to other iron drugs and is suitable for treatment of patients with increased sensitivity to GI effects, namely pregnant women and children. It also more convenient to store at home, as far as children's safety is concerned, and can be for long periods of time and is associated with insignificant adverse effects. Such observations are also important for blood transfusion services, where administration of insignificant supplements helps to preserve adequate iron pool in the body, maintaining physical and psychological well-being of donors.
Iron in the form of various drug products is registered in many countries. Ferrum Lek is a iron(III)-hydroxide polymaltose complex and has a long history of clinical use. Multiple post-marketing data of observational studies proves that iron(III)-hydroxide polymaltose complex has a favorable risk/benefit ratio and is one of the most efficient iron drugs [69, 70, 81, 82].
Despite the fact that the absorption of ferrous sulfate is more effective compared to other iron preparations, and daily increase of hemoglobin concentration is more prominent during first three weeks of therapy, iron(III)-hydroxide polymaltose complex provides equal efficiency in 9 weeks of treatment, whereas the volume of iron involvement into body processes is equal to that of ferrous sulfate.
Ferrum Lek is safe in coadministration with other drug products, which is not associated with the decrease of iron absorption. The drug is suitable for treatment of children, pregnant women, and elderly patients.
So far there have been no toxic reactions and overdose cases associated with iron(III)-hydroxide polymaltose complex.
Adverse events described in the SPC represent the range of possible adverse effects properly. Other issues related to the drug safety are described just as properly.

2.3.8 Conclusion of risks and benefits

All dosage forms of Ferrum Lek drug are indicated for treatment of iron deficiency manifestations in infants, children, adults, breastfeeding mothers, and pregnant women. Due to the fact that a low iron dose is recommended for treatment of latent iron deficiency in infants under 1 year, as well as iron deficiency prevention in infants under 1 year, children, adults, and breastfeeding mothers, tablets and syrup are not dosage forms traditionally used for such indications.
Iron(III)-hydroxide polymaltose complex is characterized by the same therapeutic action and safety profile as ferrous sulfate drugs. Conducted clinical studies were comprehensive and complied with the requirements of current clinical pharmacology. Not every study described in this review complied with the requirements of GCP. However, it does not affect the results of the review. Therapeutic efficacy of IPC seems to be appropriate to recommend this preparation as one of the most efficient preparation for additional iron therapy. Furthermore, IPC is a preparation of choice in case of high-risk categories of patients, such as preterm or newborn infants, children under eight years, and pregnant women, patients with ferrous sulfate intolerance. Cases of treatment refusal, interruption, and discontinuation are significantly less common in the case of IPC compared to other iron drugs.
Considering specific features and benefits, IPC drugs can be called a new generation of iron therapy.
Efficacy

Based on clinical use and well-controlled clinical studies, it was shown that IPC is a highly effective therapeutic drug for treatment of latent and manifesting iron deficiency forms, as well as the prevention of iron deficiency during pregnancy.
Described dosage forms have significant benefits, whereas the safety profile was improved, as iron of active substance in iron(III)-hydroxide polymaltose complex is not present in GI tract in a free form and does not get absorbed through passive diffusion.
It was proven that IPC has equal efficacy in iron-replacement therapy compared to ferrous sulfate, but it is much better tolerated.

Safety

Clinical studies demonstrated IPC safety in treatment of latent and manifesting iron deficiency forms, as well as the prevention of iron deficiency during pregnancy. Based on clinical use, there is a well-known range of adverse effects associated with the drug; these effects are predominantly minor and temporary. In general, chewable tablets and IPC syrup are well-tolerated.
Symptoms of GI tract irritation, such as abdominal fullness, pressure in epigastric region, nausea, constipation, and diarrhea, are very uncommon and present the most prevalent events. Dark stool color has no clinical significance. There were no registered toxic adverse effects or acute intoxication cases associated with the product.
Results of fecal occult blood test (hemoglobin selective) do not change when taking the drug, which makes it possible to continue treatment without interruption at the time of the test.
Ferrum Lek chewable tablets are contraindicated to patients with hypersensitivity to any of the drug components. The drug is contraindicated to patients with excessive iron content or with disorders of iron utilization processes (hemosiderosis, hemochromatosis, lead anemia, sideroachrestic anemia, thalassemia), as well as with anemia not associated with iron deficiency (hemolytic anemia).
Due to the low recommended iron dose chewable tablets are not used with children under 12 years.
Chewable tables should not also be prescribed to children under 3 years, as there is a risk of aspiration, which means that these children should take syrup instead.
Precautions should be taken when prescribing Ferrum Lek to patients with diabetes mellitus or phenylketonuria.
Oral forms of iron therapy should not be prescribed to patients receiving EPO or dialysis for the correction of iron deficiency.
In the case of pregnancy or lactation, IPC tablets or syrup can be taken only after consultation with physician.
Toxic, cancerogenic, and mutagenic action of this drug was not proven neither by means of clinical, nor epidemiological tests.
Oral iron drugs should not be prescribed simultaneously with parenteral forms.
If oral use of iron drugs was not successful, a possible reason could include incorrect diagnosis, a lack of compliance, constant bleeding, underlying infection, malignant neoplasms, liver disorders, and iron malabsorption.
So far there has been no evidence of interaction with other drug products, so interaction with food components and accompanying drug products is unlikely.
Iron therapy should not be prescribed to patients receiving repeated blood transfusion.
The SPC submitted by the applicant adequately reflects indications for use, dose descriptions, and possible risks associated with the use of the product. Adverse effects are described together with observations made during clinical studies.
Latest data support the IPC use for treatment and prevention of iron deficiency in infants, children, and pregnant women.

2.4 Potential risk and benefit of the investigational drug products for the study participants

List of known adverse events associated with Ferrum Lek® (iron(III)-hydroxide polymaltosate) chewable tablets, 100 mg, manufactured by Lek d.d. (Slovenia) as compared with reference product Maltofer® (iron(III)-hydroxide polymaltosate), 100 mg chewable tablets (Vifor S.A., Switzerland) is present in Sections 2.1.1 and2.1.2, respectively.
Clinical study will be conducted according to the protocol of theclinical study and the rules of Good Clinical Practice (decree of the Ministry of Health of the Russian Federation No. 200n dated 01.04.2016 "On Approval of Good Clinical Practice Rules", National standard of the Russian Federation GOST R 52379-2005 "Good Clinical Practice"), treatment of patients will be conducted by qualified healthcare personnel based on current clinical recommendations on treatment of patients with IDA. Treatment and examination during the study will be free of charge for the patients. Therefore, potential benefit of participating in the study exceeds potential risk of using the study and reference products.

2.5 Description and rationale for the route of administration, dosing regimens, and duration of treatment

The study drugs will be prescribed according to relevant Product Information:
Ferrum Lek® drug will be used according to the following scheme: 200 mg (2 tablets) with or immediately after meal to be taken as a single dose for 84 days.
Reference product Maltofer® will be used according to the following scheme: 200 mg (2 tablets) with or immediately after meal to be taken as a single dose for 84 days.

2.6 Description of the study population

Adult patients of both sexes (18 years and older) with IDA and indication of iron therapy.

2.7 Legal framework for the conduction of clinical study

This is a protocol of the clinical study planned to be conducted in accordance with principles of the Declaration of Helsinki, World Medical Association (adopted at the 18th Assembly of the World Medical Association in Helsinki in June 1964, the latest revision adopted by the 64th Assembly in Fortaleza in October 2013), the triangular agreement on Good Clinical Practice (GCP, ICH E6(R2) dated 09.11.2016), and applicable laws of the Eurasian Economic Union and the Russian Federation:

- Rules for Registration and Expertise of Medicinal Products for Medical Use (adopted by Decision No. 78 of the Council of Eurasian Economic Commission dated November 3, 2016);
- Guideline for Good Clinical Practice of the Eurasian Economic Union (adopted by Decision No. 79 of the Council of Eurasian Economic Commission dated November 3, 2016);
- Federal Law No. 61-FZ "On Circulation of Medicines" dated 12.04.2010 (revision valid from January 1, 2017 with latest changes according to Federal Law No. 350-FZ dated July 3, 2016);
- Federal Law No. 152-FZ "On Personal Data" dated 27.07.2006 (revision valid from July 2017 with latest changes according to Federal Law No. 148-FZ dated 01.07.2017);
- Decree of the Ministry of Health of the Russian Federation No. 200n dated April 1, 2016 "On Approval of Rules for Good Clinical Practice";
- The national standard of the RF GOST R 52379-2005 "Good Clinical Practice".

- Government Decree of the Russian Federation No. 714 of September 13, 2010 (with latest changes and updates in revision dated October 15, 2014) "Rules for Compulsory Insurance of the Life and Health of a Patient Involved in Clinical Trials of a Drug";
- Order of the Ministry of Health of the Russian Federation On Approval of the Provision on the Ethics Council No. 986n dated November 29, 2012.

3 Study goals and objectives

3.1 Study purpose

The purpose of this study is to test the hypothesis of non-inferior efficacy and safety of Ferrum Lek® (iron (III) hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia), as compared with MALTOFER® (Vifor S.A., Switzerland), in subjects with mild and moderate iron deficiency anemia.

3.2. Study objectives

Main objective:

- To assess the non-inferiority of the therapeutic efficacy of Ferrum Lek® 2 tablets daily (200 mg), via assessing the effect on hemoglobin level in the blood (g/l) after 12 weeks of treatment for patients with iron-deficiency anemia of mild and moderate severity compared with MALTOFER® 2 tablets daily (200 mg) during the same period.

Secondary objectives:

- Assessment of safety of Ferrum Lek® 2 tablets daily (200 mg), compared with MALTOFER® 2 tablets daily (200 mg), via the frequency, characteristics, intensity and relationship to treatment received of AEs.

4 Study design

4.1 Primary and secondary evaluated parameters

***Primary efficacy endpoint**:*

- Changes in blood haemoglobin level (g/L) after 12-weeks of iron-deficiency anaemia treatment, a non-inferiority comparison, as compared with the baseline value (screening visit) between Ferrum Lek® and MALTOFER® groups.

Secondary efficacy endpoints:

- Absolute values and changes of blood hemoglobin level (g/L) after 4, 8 and 12-weeks of treatment in the study groups.
- Changes in average values of iron metabolism parameters (ferritin, transferrin, percent transferrin saturation, serum iron) after 4, 8 and 12 weeks of iron-deficiency anaemia treatment, as compared with the baseline value in the study groups.
- The frequency of response to therapy, determined as an increase in haemoglobin level by 20 g/L and more after 12-weeks of iron-deficiency anemia treatment in the study groups.
- Frequency and severity of all adverse events observed during the study therapy in the study groups.

Safety Measurements
Safety evaluation will include:
1) Determination of the total number, frequency and severity of:
   I. adverse events (AEs) irrespective of their relation to treatment;
   II. AEs related or possibly related to the treatment received;
   III. AEs requiring termination of the therapy.
2) Complaints on digestive system disorders (defined through MedDRA SMQ for the corresponding symptoms), considered as related to the study therapy by the investigator, will be analyzed separately.
Descriptive statistics will be used for presentation of the result. Comparison of all AE incidence between the study groups will be performed using the Fisher's exact test or chi-square test depending on the number of expected observations per cell (<5 or ≥5), and comparison of intensity (and possible causal relationship between the treatment received and the AE) will be performed using Cochrane-Armitage trend test for the ordered categorical data.
3) Changes in laboratory test results (assessed in central laboratory) over time and frequency of abnormal test results (according to the normal ranges of central laboratory) will be summarized by group and also compared by group using corresponding tests for quantitative data and qualitative data (see below).
4) Changes in vital signs over time and frequency of abnormal physical examination findings will be summarized by group and also compared by group using corresponding tests for quantitative data and qualitative data (see below).
Quantitative data will be compared using the t-test or Mann-Whitney test depending on the normality of the distribution (which will be tested using Shapiro-Wilk test).
Qualitative data will be compared using the Fisher's exact test or chi-square test depending on the number of expected observations per cell (<5 or ≥5).

4.2 Study design description and flowchart

4.2.1 Number of centers and patients

20 study sites in the Russian Federation will participate in the study; they will treat and monitor 336 patients.

4.2.2 Study population

Study population – 336 adult outpatients of both sexes with diagnosed mild and moderate iron-deficiency anemia (hemoglobin level below 110 g/L (in men and women), but above 80 g/L), who are iron-naive patients.

4.2.3 Design and treatment description

Phase III multicenter randomized open-label prospective comparative parallel active-control study (in the Russian Federation). Upon signing of the informed consent for participation in the study, the subjects will undergo screening for up to 7 days (Visit 0). The eligible and ineligible subjects will be randomized into two treatment groups at the ratio 1:1:
The subjects in the first group (168 subjects) will receive Ferrum Lek® chewable tablets – 2 tablets daily (200 mg), during or right after meals; the daily dose should be administered once daily (as a single dose). The subjects in the second group (168 subjects) will receive MALTOFER® chewable tablets – 2 tablets daily (200 mg), during or right after meals; the daily dose should be administered

once daily. Subjects will take the products daily during 12 weeks and fill out a diary (see Appendix 16.1), where they will register administration of the study drugs and concomitant therapy.
All subjects will undergo a complete blood count and blood biochemistry. Therapy under investigation also includes an interim Visit 2 (Day 29 ± 2), Visit 3 (Day 57 ± 2), and final Visit 4 (84 ± 2). After the final visit a subject's participation in the study shall be terminated, and further treatment shall be performed by an attending physician.
Study design is depicted in Figure **1**.

4.2.4 Duration of the study

Total duration of the study will be no more than 12 months and will include subjects recruitment (9 months), treatment (3 months) and a Follow-up visit (by phone) 14 days after the end of the active therapy period.

4.2.5 Study type

Phase III multicenter randomized open-label prospective comparative parallel active-control study (in the Russian Federation). Randomization is a measure aimed at minimizing subjectivity due to random allocation of subjects. Randomization excludes subjective, as well as unperceived allocation of population, which differs significantly from the population of the second group, into one group by the Investigator.
A study of non-inferior efficacy and safety of Ferrum Lek® (iron-III-hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia), compared to MALTOFER® (Vifor S.A., Switzerland), for treatment of mild and moderate iron deficiency anemia in adults, will be conducted.

4.2.6 Study design flowchart

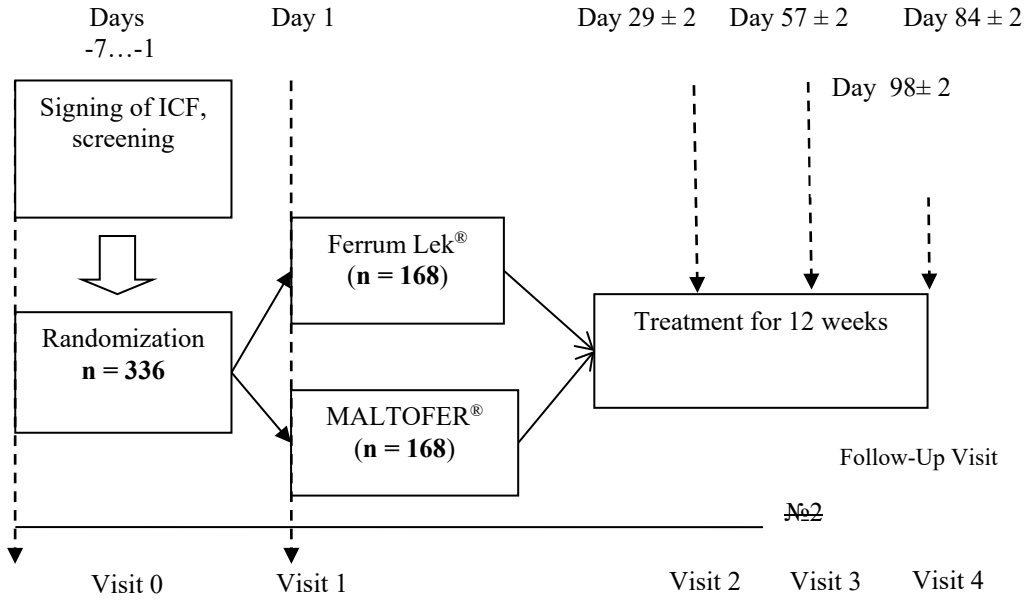

Figure 1 Study design flowchart

4.2.7 Visit schedule

After the randomization during Visit 1 subjects will be asked to take the drug daily on their own (Ferrum Lek® or MALTOFER®) and make a Visit 2 (day 29 ± 2), Visit 3 (day 57 ± 2), and Visit

4 (day 84 ± 2). After the last planned visit to the study site, a Follow-up visit (by phone) is to be made 14 days after the end of the active therapy period for the registration of delayed adverse events (Day 98 ± 2).
Patients can make unplanned visits at the center, if they need medical intervention, at the discretion of an investigating physician.
Table **5** contains visit and procedure schedule.

Table 5 Schedule of study procedures

Study periods	**Screening**	**Randomization and start of the treatment**	**Treatment**		
Visit No. / days	**Visit 0** Days -7…0	**Visit 1** Day 1	**Visit 2** Day 29±2	**Visit 3** Day 57±2	**Visit 4** Day 84±2
Study procedures					
Signing of the informed consent	X				
Inclusion/non-inclusion criteria	X	X			
Demographic and baseline data [2]	X				
Vitamin B_{12} (cyanocobalamin) level	X				
Vitamin B_9 (folic acid) level	X				
TSH level	X				
T_4 level	X				
The history of iron-deficiency anemia and significant concomitant diseases	X				
Physical examination, BP and heart rate measurement	X		X	X	X
Urinalysis [3]CL	X				X
Urine pregnancy test	X				X
HIV antibody testing [4]CL	X				
Blood chemistry [5]CL	X				X
Complete blood count [6]CL	X		X	X	X
Iron metabolism parameters [7]CL	X		X	X	X
Creatinine index[8]					
Randomization		X			
Start of therapy with the study drugs		X			

[1] Follow-up visit will be conducted by contacting patient by phone to find out possible AEs.
[2] Date of birth, sex, body weight.
[3] General properties (color, pH, specific gravity, protein) and sediment microscopy.
[4] Except for the subjects with HIV test results obtained within the last 6 months.
[5] Total protein, total and conjugated bilirubin, AST, ALT, alkaline phosphatase, blood creatinine, glucose, CRP.
[6] At screening – hemoglobin, hematocrit, RBC, PLT, WBC, WBC differentiation, ESR. During the Visit 2, 3 and final visit – hemoglobin, hematocrit, RBC.
[7] Serum ferritin, serum iron, transferrin, percent transferrin saturation.
CL – Central laboratory
[8] For <u>males</u> (reference range 90–150 mL/min):

$$GFR = 1.23 * \frac{\big(140 - age\ (years)\big) * weight\ (kg)}{blood\ creatinine\ (\frac{umol}{L})}$$

For fe<u>males</u> (reference range 90–130 mL/min):

$$GFR = 1.05 * \frac{\big(140 - age\ (years)\big) * weight\ (kg)}{blood\ creatinine\ (\frac{umol}{L})}$$

Handing out of a subject's diary and fill-in instructions		X	X	X		
Check of a subject's diary completion and collection of diaries			X	X	X	
Keeping a subject's diary		daily				
Accounting and distribution of the study drugs		X	X	X	X	
Compliance analysis			X	X	X	
Concomitant therapy	X	X	X	X	X	
Exclusion criteria		X	X	X	X	
Recording of adverse events	X	X	X	X	X	

4.2.8 Study procedures

4.2.8.1 Collection of complaints, medical history, demographic and anthropometric data

When collecting history, particular attention is paid to the history of iron deficiency anemia (IDA):

- Investigator will register primary diagnosis which led to the development of IDA (ICD-10), if present, as well as the cause and duration of IDA (from the moment of diagnosis). Registration of previous IDA treatment with iron drugs or blood transfusion (if applicable);
- IDA causes, is applicable, should be classified to:
    - Vast blood loss (polymenorrhea, uterine myoma, intrauterine contraceptive, abortion, endometrial polyps, menorrhagia of different genesis, hemorrhoidal bleeding, nasal bleeding, GI bleeding);
    - Donation;
    - Alimentary iron deficiency;
    - Other (if applicable).

Patient is asked about previous diseases, concomitant chronic diseases, hereditary issues, social habits (smoking, alcohol consumption, drug consumption). History of allergies, previous surgeries or traumas, professional history, permanent or periodic drug therapy. A contraception method used by the patient is specified; by signing the informed consent, the patient agrees to use one of the recommended contraception methods (by the protocol) during the study and 1 month after the study completion.

Primary documents must contain data on the sex, age, race, and ethnicity of the patient. A height meter is used to measure the patient's height in centimeters, the weighing scales—body weight.

The anthropometric examinations will be conducted in accordance with the Health Risk Monitoring (EHRM) Recommendation for indicators, international collaboration, protocol and manual of operations for chronic disease risk factor surveys, 2002 (European Health Risk Monitoring (EHRM), 2002).

Height should be measured in all the patients (the causes which make the height measurement impossible include hair-style peculiarities (e.g., mohawk), patient's refusal to take off a headwear (e.g., turban), patient's incapacity to stand, exceedance of the height meter's scale). The height meter is a vertically positioned panel with a centimeter division with a sliding horizontal ruler. A standard error must not exceed 2 mm. The patient will be asked to take off his/her shoes, thick clothes, hairpins, or other hair accessories. The patient must touch a vertical panel with his/her hindhead, back, buttocks, calves, and heels (the toes are stuck together). Upper boundary of the external ear canal must be at the same level with lower boundary of the orbital cavity (the jugal bone). The patient is asked to look forward. If the patient's height exceeds that of the Investigator, the latter should use a platform. A height meter's ruler is put on the patient's head, the scale's divisions are counted. The height must be recorded while the patient is still standing under the ruler. An error must not exceed 0.5 cm.

The patient's weight must be measured in the morning in the fasting condition, after urination and defecation, in the underwear (with further subtraction of an average underwear weight). The weighing scales must be horizontally positioned on a solid ground. An error must not exceed 0.2 kg. If the weight measurement is impossible, the Investigator should not record any values based on the patient's claims.

BMI will be calculated according to the formula:

$$\text{BMI} = \text{body weight in kg} / \text{height in m}^2$$

Rounding to decimals will be done according to the standards of mathematical rounding.

4.2.8.2 Assessment of the vital signs

During the examination before study (screening) and during each visit, systolic and diastolic blood pressure, pulse rate must be measured on the non-dominant arm after a 5-minute rest in the sitting position. Respiratory rate shall also be measured.
Body temperature must be measured using the same method (axillary temperature) throughout the study.

4.2.8.3 Physical examination

Physical examination will be conducted according to the general rules of the propedeutics of internal diseases: general visual examination, inspection of the mucous membranes, lymph nodes palpation, evaluation of bones and muscles, palpation, percussion, and auscultation of the main organ systems (cardiovascular, respiratory, gastrointestinal, urinary systems) will be conducted sequentially.

4.2.8.4 Laboratory tests

The blood samples for analysis will be collected in the morning, in the fasting state (8–10 hours after the last meal) from the cubital vein by a disposable sterile syringe under aseptic conditions. Approx. 12 ml of blood are necessary for the test. For the urinalysis patient collects morning midstream urine after proper hygiene of perineum. Blood samples for hematology test, blood chemistry, as well as urine samples for urinalysis are taken during Visit 0 (screening) and 3.
Hematology test (hemoglobin level, hematocrit, RBC, PLT, WBC, leucogram, ESR), blood biochemistry (total protein, total and conjugated bilirubin, AST, ALT, alkaline phosphatase, blood creatinine, glucose); iron metabolism evaluation (ferritin, transferrin, iron transferrin saturation percentage, serum iron), and urinalysis (general properties (color, pH, specific gravity, protein) and sediment microscopy) will be conducted in Invitro central laboratory.
Results of hemoglobin measurement must be interpreted according to the classification of WHO and A.A. Miterev:

- Hemoglobin 110-90 g/L – mild anemia;
- Hemoglobin 89-70 g/L – moderate anemia;
- Hemoglobin <70 g/L – severe anemia.

After obtaining blood creatinine results, blood creatine will be calculated using the Cockroft-Gault Formula [97]:
For males (reference range 90– 150 mL/min):

$$GFR = 1.23 * \frac{\big(140 - age\ (years)\big) * weight\ (kg)}{blood\ creatinine\ (\frac{umol}{L})}$$

For females (reference range 90– 130 mL/min):

$$GFR = 1.05 * \frac{\big(140 - age\ (years)\big) * weight\ (kg)}{blood\ creatinine\ (\frac{umol}{L})}$$

Vitamin B_9 (folic acid) and vitamin B_{12} (cyanocobalamin) blood tests will be done at the screening visit to exclude vitamin B_{12} and folic acid deficiency.
TSH and T_4 blood test will be conducted at the screening visit to exclude thyroid dysfunction.
HIV antibodies tests will be conducted during screening of patients without the results of HIV test, made within last 6 months; samples will be sent to Invitro central laboratory.
Urine pregnancy test with a dipstick must be performed for all women with the preserved childbearing potential at the screening visit (pregnancy test is not necessary for women with

documented hysterectomy and/or bilateral ovariectomy and/or tubal ligation, as well as for women in postmenopause [with menostasia for more than 1 year, with the corresponding clinical profile]).

4.2.8.5 Randomization

Upon signing of the informed consent for participation in the study, the subjects undergo screening for up to 7 days. The eligible and ineligible subjects will be randomized into two treatment groups at the ratio 1:1, distribution method is described in Section 4.4.

4.2.9 Visit objectives

4.2.9.1 Visit 0 (screening, days -7...-1)

Visit objective:

- Signing of the informed consent;
- Assessment of inclusion/exclusion criteria;
- Collection of demographic and anthropometric data;
- Collectopn of history of iron deficiency anemia and significant concomitant diseases;
- Physical examination, blood pressure and heart rate measurement;
- Laboratory tests:
    - Urinalysis (general properties (color, pH, specific gravity, protein) and sediment microscopy);
    - Urine pregnancy test;
    - HIV antibodies test, except for subjects who had an HIV test within last 6 months;
    - Blood chemistry (total protein, total and conjugated bilirubin, AST, ALT, alkaline phosphatase, blood creatinine, glucose, CRP);
    - Complete blood count (hemoglobin level, hematocrit, RBC, PLT, WBC, leucogram, ESR);
    - Metabolic parameters of iron (serum ferritin and iron level, transferrin, iron transferrin saturation percentage);
    - Vitamin B_9 (folic acid) and vitamin B_{12} (cyanocobalamin) blood levels;
    - TSH and T_4 blood levels;
    - Calculation of creatinine clearance using the Cockroft-Gault Formula;
- Evaluation of the concomitant therapy;
- Adverse events registration.

4.2.9.2 Visit 1 (randomization / start of the treatment, day 1)

Visit objective:

- Assessment of inclusion/exclusion criteria;
- Randomization;
- Start of the treatment with the study product;
- Hand-out of the patient diary (Appendix16.1) and instructions on its completion;
- Accounting and dispensing of the study product;
- Evaluation of the concomitant therapy;
- Adverse events registration.

4.2.9.3 Visit 2 (continuation of the treatment, day 29±2)

Visit objective:

- Physical examination, blood pressure and heart rate measurement;
- Metabolic parameters of iron (serum ferritin and iron level, transferrin, iron transferrin saturation percentage);

- Complete blood count (hemoglobin level, hematocrit, RBC);
- Hand-out of the patient diary (Appendix16.1) and instructions on its completion;
- Check of a patient diary completion and collection of diaries;
- Accounting and dispensing of the study product;
- Evaluation of compliance;
- Evaluation of the concomitant therapy;
- Adverse events registration.

4.2.9.4 Visit 3 (continuation of the treatment, day 57±2)

Visit objective:

- Physical examination, blood pressure and heart rate measurement;
- Metabolic parameters of iron (serum ferritin and iron level, transferrin, iron transferrin saturation percentage);
- Complete blood count (hemoglobin level, hematocrit, RBC);
- Hand-out of the patient diary (Appendix16.1) and instructions on its completion;
- Check of a patient diary completion and collection of diaries;
- Accounting and dispensing of the study product;
- Evaluation of compliance;
- Evaluation of the concomitant therapy;
- Adverse events registration.

4.2.9.5 Visit 4 (efficacy and safety evaluation, day 84±2)

Visit objective:

- Physical examination, blood pressure and heart rate measurement;
- Laboratory tests:
    - Urinalysis (general properties (color, pH, specific gravity, protein) and sediment microscopy);
    - Urine pregnancy test;
    - Blood chemistry (total protein, total and conjugated bilirubin, AST, ALT, alkaline phosphatase, blood creatinine, glucose, CRP);
    - Complete blood count (hemoglobin level, hematocrit, RBC);
    - Metabolic parameters of iron (serum ferritin and iron level, transferrin, iron transferrin saturation percentage);
- Check of a patient diary completion and collection of diaries;
- Accounting and dispensing of the study product;
- Evaluation of compliance;
- Evaluation of the concomitant therapy;
- Adverse events registration.

4.2.9.6 Follow-up visit (Visit 5, day 98 ± 2)

The follow-up visit is conducted in the form of contact with the patient by phone to determine the state of health and the presence of adverse events. This visit is performed 14 days after the EOT visit.

Follow-up visit procedures (by phone):

• Adverse events assessment.

If there are any adverse events, the patient may be invited to the site for an unplanned visit.

4.2.10 Early study termination

Sponsor has the right to terminate the study, and the Investigator has the right to stop subjects recruitment at any time. In the case of premature closing of the center/study, all completed, as well as unused CRFs (including unused pages of partially completed CRFs) and other documents (excluding documents, which must be kept in the centre) must be returned to the Sponsor. Study materials can be destroyed only with the consent of the Sponsor.

4.3 Handling of the investigational drugs

4.3.1 Delivery of drugs to the center

The Sponsor provides Ferrum Lek® and MALTOFER® to the study site. The Sponsor is responsible for manufacture, packaging, labelling, and delivery of drugs, specification of storage conditions and drug's shelf-life. Primary and secondary packaging of drugs will contain the label "For Clinical Study Only".
The Investigator or an authorized person will receive the delivery of the investigational products at each study site. drugs must be stored in a restricted area, where the temperature corresponds to that of storage conditions. Each delivery of drugs must be confirmed by means of acceptance note. The Investigator is responsible for the proper drug storage, its administration to the study participants and accounting. In the case of violation of drug storage conditions, as well as the observation of flaws or damage of investigational drugs or their packaging upon delivery to the research center, the Investigator must immediately notify the Monitor of the study.

4.3.2 Quality complaints

4.3.2.1 Definitions

Claims for the investigational drug product (study product and reference product) or medical device may include:

1) Claims related to the quality of the product/medical device such as

- any fault of quality and/or efficacy e.g. change of visual appearance, change of amount, damaged tablets/capsules, presence of particulate matter.
- any fault of the containers and outer packages e.g. surface defect, container leakage, broken syringe/plunger, missing contents, device malfunction.
- any fault of the labeling e.g. missing or illegible label.
- any falsification of the medicinal product or medical device e.g. suspected product mix-up, tampering or counterfeiting.

2) Complaints related to the transportation of the product, such as damaged packaging for transport and/or damaged secondary packaging after receipt of the goods, a lack of quantity of medicinal product in the packaging for transport, inadequate transport conditions.

4.3.2.2 Procedure

In case any of the complaints listed above are detected or information about these complaints has been received, the completed Non-conformance Report must be sent to the Sponsor (study manager/Clinical Research Manager) within 24 hours. Simultaneously local monitor or CRA of the respective study must be informed about the complaint. If possible, a photo of the affected material should be attached to the report. Affected material should be retained and stored according to the storage conditions label and/or returned to sponsor if requested by the Sponsor.

4.3.3 Dispensing and return of drugs

Investigational products are intended only for the study subjects and must be used according to the protocol.
Investigational products will be dispensed to patients during each visit in the quantities calculated until the next visit. Patients will be asked to return all used and unused drug packages to the center upon each visit.

4.3.4 Drug records

The Investigator or a designated person must keep records of all the drugs in the center during the study by means of completing record forms for the drugs provided by the Sponsor. Records of investigational drugs will be controlled by the Monitor regularly.
The Investigator or any designated person will count the number of packages of used or unused drugs returned by the patient and enter this data into the record forms for the drugs in CRFs. Moreover, CRFs will contain the number of missing visits, investigational product dose changes (if any, according to the patient diary).

4.3.5 Return and destruction of drugs

Upon completion of the study or in case of early study termination on the part of the study site, all unused investigational products and packages of used preparations must be returned to the Sponsor. The Sponsor is responsible for the destruction of investigational products. After the counting of all the packages of used and unused drugs and control of the packaging labels, the Monitor organizes drug shipping to the Sponsor for the following destruction.

4.4 Subjectivity minimization measures

4.4.1 Randomization

A randomized open prospective comparative phase III trial in parallel groups with active control is planned.
Randomization is a measure aimed at minimizing subjectivity due to the random distribution of the patient. Randomization will exclude the subjective, including unconscious, selection by the researcher in one of the patient population groups, which will differ significantly from the population recruited in the second.
Automated randomization lists will be formed, randomization will be carried out through the e-CRF system with a randomization module.
Each randomized patient will be assigned a unique number for issuing the drug during the study. The patient's number consists of the letter R and the numbers from 001 to 336. The numbering of patients does not depend on the research center and is “transparent”. In addition to the number, the system will indicate the treatment group to which the patient is distributed. Randomization will be stratified by two factors depends on patient’s data (gender and baseline Hb level) entered to the e-CRF before randomization:

- Gender (male vs female);
- Hb level (80–94 g/L vs 95–110 g/L).

In order to randomize patients in the e-CRF system, each center will be provided with secure access and appropriate training in the use of the system will be provided.

The randomization number and name of the treatment group issued by the system. The researcher records in the primary documentation and enters information on the randomized patient in the list of included patients. The randomization number cannot be changed during the study.
The drug is given to the patient according to the information on the group of therapy received from e-CRF system. Research therapies also cannot be changed during the research process.

4.4.2 Blinding

The study treatment is prescribed openly. Blinding within this study is not planned.

4.5 Dosing schedule for investigational products

Study product Ferrum Lek® (iron-III-hydroxide polymaltosate), 100 mg chewable tablets (Lek d.d., Slovenia), 2 tablets daily (200 mg), during or right after meals; the daily dose should be administered once daily.
Reference product MALTOFER® (Vifor S.A., Switzerland) (iron-III-hydroxide polymaltosate), chewable tablets, 2 tablets daily (200 mg), during or right after meals; the daily dose should be administered once daily.

4.6 Dosage form, package and labeling of the investigational products

4.6.1 Dosage form

Investigational products: Ferrum Lek®, 100 mg chewable tablets (Lek d.d., Slovenia). Dark brown round flat tablets with light brown impregnations and a bevel.
Reference product: MALTOFER® (Vifor S.A., Switzerland) 100 mg chewable tablets. Dark brown, round, flat scored tablets with white impregnations.

4.6.2 Container

Investigational products: 100 mg chewable tablets. 10 tablets per strip or blister. 3, 5 or 9 strips or blisters in a carton pack along with the Patient Information Leaflet.
Reference product: 100 mg chewable tablets. 10 tablets in polyethylene laminated aluminum foil blisters. 1 or 3 blisters per carton pack along with the Patient Information Leaflet.

4.6.3 Labeling of the study drugs

This study is open-label, the study site will be provided with the packed drug products from the series commercially available.

Each package, in addition to the commercial label with the name, composition, shelf life and drug product manufacturer, will be labeled with a sticker containing following information: note "For Clinical Trials Only", clinical study protocol number, name and contacts of the study Sponsor and Investigational Site, and place for writing patient' randomization number.

The label should not cover the drug name, active substances, and their strength, shelf life, manufacturer name.

4.7 Duration of patients participation in the study

Duration of each patient participation in the study will be not longer than 98 days (including screening, 7 days; randomization and treatment, 84 ± 2 days).
Minimal duration of patients participation in the study: 83 days (on condition of minimally acceptable treatment duration and screening duration, 1 day).

4.8 Rules of termination of clinical study parts or the study on the whole

The study may be terminated on the following grounds:
1. Upon the Sponsor's initiative:
   a. availability of new toxicological or pharmacological data, or data on SAE which require re-consideration of the previous evaluation of the benefit/risk ratio.
   b. AEs incidence or severity require the study termination;
   c. other reasons including administrative ones.
2. Upon the Investigator's initiative: AEs incidence or severity unacceptably increases risk of patients participation in the study.
3. Upon the decision of the regulatory authorities.

In the case of early termination of the study, the Sponsor must inform the study site personnel as well as regulatory authorities with indication of the reason thereof.

4.9 Record keeping procedures for investigational drug products

The study sites will be provided with the investigational product (the study product and reference product) in the amounts sufficient for the study completion, taking into account the planned number of patients to be screened.
An authorized study site specialist shall keep a logbook for the drugs where the drugs delivery to the study site, dispensing to the subjects, and return of the unused drugs are noted. Storage conditions must be also noted. The study drugs may be used for the purposes of this clinical study only.
Authorized representatives of the Sponsor or regulatory authorities may check the drugs log or availability of the drugs at stock during the study site audit/monitoring. The drugs must be stored under the required conditions in a room with an access for the authorized personnel responsible for the drugs dispensing only.

4.10 Storing and unblinding of randomization codes

The table with treatment randomization codes will be stored in Sponsor's company, the access to this information will be restricted to designated persons. Procedures of unblinding of randomization codes are not applicable within this study, as the blinding of treatment groups for this study is not planned.
Randomization plan will be known to the investigating physician, the Principal Investigator, the Sponsor, the regulatory authorities (in observation of serious adverse events).

4.11 List of data registered in Case Report Form (without preliminary written or electronic record) and recognized as primary data

All study data will be entered into primary documentation and CRFs.

5 Selection and exclusion of patients

5.1 Inclusion criteria

Patients will be included into the study, if they comply with the following criteria:

1) The signed and dated written informed consent prior to participation in the study.
2) Men and women aged 18 and older (by the time of screening).
3) Outpatients.
4) Diagnosed iron-deficiency anemia, based on two criteria:
   a) hemoglobin level below 110 g/L (in men and women), but above 80 g/L,
   b) serum ferritin levels below 30 μg/L.
5) Women are eligible for enrollment if they are:
   a) **not fertile** (i.e., women in postmenopause or after surgical sterilization). Surgically sterile women are considered as female subjects with documented hysterectomy and/or bilateral ovariectomy and/or tubal ligation. Women in postmenopause are considered as women with menostasia for more than 1 year, with the corresponding clinical profile, e.g. older than 45 years, in absence of hormonal replacement therapy. However, in case of doubt, a blood sample shall be taken, where FSH content shall be above 40 IU/ml, and estradiol content – below 40 pg/ml (below 140 pmol/L), in order to confirm postmenopause.

   OR

   b) **fertile**, but the result of pregnancy test at screening is negative, and the subject agrees to use one of the following contraception methods constantly and properly (i.e. in accordance with the approved prescribing information and the doctor's orders) during the whole period of participation in the study:
      i) Total sexual abstinence
      ii) Oral contraceptives (combination drugs containing progestogen, or progestogen alone)
      iii) Injectable progestogen
      iv) Levonorgestrel implants
      v) Estrogen-containing vaginal ring
      vi) Transdermal contraceptive patches
      vii) Intrauterine device or intrauterine system
      viii) A male partner has been sterile (vasectomy with documented azoospermia) **prior to enrollment of a woman**, provided that he is the only partner of the female subject. For the purpose of this definition, "documented" is related to the result of a subject's medical examination by an investigator/responsible person or a subject's past medical history review for assessment of eligibility for enrollment, obtained during the interview with a subject or from his/her medical records.
      ix) Double barrier method: a condom or an occlusive cap (diaphragm or cervical/vault caps) with a spermicide (foam/gel/film/cream/suppository).

5.2 Non-inclusion criteria

Patients cannot take part in the study if they comply with any of the following criteria:
1) Administration of any iron-containing drugs during the last 3 months.
2) History of erythropoietin drugs administration.
3) Hypersensitivity to iron therapy (both Oral and/or IV administration) and other components of the study drugs.

4) Hormone therapy (including the use of androgens/anabolic steroids) or administration of drugs that inhibit blood formation, less than 3 months before the start of the study.
5) History of severe allergic reactions or drug intolerance.
6) Fructose intolerance, glucose-galactose malabsorption syndrome, and sucrase-isomaltase deficiency.
7) Pregnant or lactating women, or women intending to become pregnant during the study.
8) Failure of iron therapy for iron-deficiency anaemia in a subject's past medical history.
9) Heme metabolism disorders (e.g., sideroachrestic anaemia, lead anaemia, thalassaemia).
10) Iron overload including haemochromatosis and hemosiderosis
11) Other causes of anemia, apart from iron deficiency, including:
    a) Haemolysis (determined as per analyses results at screening, or as per anamnestic data),
    b) Vitamin B_{12} and folic acid deficiency (as per the screening data),
    c) Chronic kidney disease (creatinine clearance at screening is below 90 ml/min (based on Cockcroft-Gault Formula)),
    d) Systemic connective tissue diseases, chronic infectious diseases requiring regular therapy (as per the past medical history), and other conditions which may, in the investigator's opinion, be accompanied by anaemia of chronic diseases.
12) Dysfunction of the thyroid gland (based on the data obtained at screening).
13) Laboratory and clinical signs of an active inflammatory process for 10 days before screening.
14) AST, ALT, and total bilirubin levels exceeding the upper limit of normal 1.5 times and more.
15) Clinically apparent hypothyroidism, in the investigator's opinion.
16) Malignant diseases, including blood and lymphoid tissue disorders (leukemia, Hodgkin disease, myelodysplastic syndrome, myeloma, etc.) at screening or in the past medical history, provided that the remission was less than 5 years before screening.
17) Signs of bone marrow aplasia at screening or history of bone marrow aplasia.
18) The necessity of parenteral iron therapy, i.e. the following cases:
    a) impaired absorption in case of an intestinal pathology (enteritis, coeliac disease, malabsorption, small intestinal resection, stomach resection, including the duodenum);
    b) exacerbation of gastric or duodenal ulcer;
    c) the necessity of quick iron saturation, e.g. in patients with iron-deficiency anaemia with upcoming surgery;
    d) continuous vast blood loss and other causes, at the discretion of the investigator.
19) Known presence of an active infection caused by Helicobacter pylori. In case of presence of Helicobacter pylori, a subject may be enrolled after eradicative therapy.
20) Concomitant diseases and conditions, which, in the investigator's opinion, pose risk to a subject's safety in case of his/her participation in the study, or able to affect the safety data analysis in case of exacerbation of this disease/condition during the study, including:
    a) Myocardial infarction or stroke within 6 months before screening.
    b) Unstable angina;
    c) Severe arrhythmia, not controlled by drug therapy;
    d) Decompensated diabetes mellitus;
    e) Nephrological disorders;
    f) Other significant diseases, at the discretion of the investigator.
21) HIV infection (as per the screening data or the results of analysis performed within 6 months before screening).
22) Known or suspected drug or alcohol abuse for the last 2 years.
23) Suspected poor adherence of a subject (e.g., due to mental disorders).
24) Participation in any clinical drug studies less than 3 months before the study.
25) Blood donation/blood transfusion within 30 days prior to screening or planned blood transfusion at time of screening.

26) History of smoking, unless leave off smoking > 6 months.

)

5.3 Exclusion criteria

5.3.1 Terms and conditions for the subjects exclusion from the study

The subjects may be excluded from the study on their own free will at any moment, without giving any reason, as well as upon decision of the Investigator or Sponsor in cases when continuation of the participation may inflict harm to the patient's health and/or life. In addition, the patient must be excluded in the cases listed below:

1. Negative trend of the disease
2. The Ethics Committee, regulatory authorities or Sponsor terminate the study or participation of the specific study site for any reason.
3. The Investigator's decision to withdraw the patient from the study in the interests of the patient.
4. Withdrawal of the informed consent (unwillingness of the patient to continue his/her participation in the study).
5. Serious deviation from the study protocol;
6. Individual intolerance of study drugs;
7. Clinically significant adverse event or serious adverse event related to investigational drug;
8. Patient's non-compliance;
9. False inclusion (e.g., the patient was included in breach of the inclusion/non-inclusion criteria);
10. The patient complies with the non-inclusion criteria during the study;
11. Patient receives/needs additional treatment, which may influence the study results or patient's safety;
12. Other conditions or events which, in the Investigator's opinion, require the patient's exclusion from the study.

5.3.1.1 Terms and volume of data on the excluded subjects

In the case of study discontinuation on the subject's free will, the Investigator should make effort to find out the reason thereof. In the case of study discontinuation due to AEs/SAEs, the subject should be followed-up until the AEs/SAEs are resolved. In case of early study termination by a patient, he/she should visit the study site for early termination procedures (as an unscheduled visit); if the patient refuses to visit the study site, he/she should be contacted by phone with further recording of all available information.

5.3.2 Terms and conditions for suspension or discontinuation of the study therapy

The patients may stop taking the study drugs according to the protocol or upon withdrawal from the study.

5.3.3 Replacement of the excluded subjects

Replacement of the excluded patients during the study is not provided for.

6 Information on drug products used in this clinical study

6.1 Administration of study and reference products

Study product (Ferrum Lek®): the daily dose of 200 mg (2 tablets) shall be taken once a day with or immediately after a meal.
Reference product (MALTOFER®): the daily dose of 200 mg (2 tablets) shall be taken once a day with or immediately after a meal.

6.2 Duration of treatment:

Patients in both groups will take drugs daily during 12 weeks.

6.3 Compliance control

Compliance will be controlled by means of checking patient's diary and accounting the amount of left drug.

6.4 Permitted concomitant therapy

Patients will take medications, which they took before the enrollment, for the treatment of concomitant diseases during the study. Women that take oral contraceptives may continue taking them during the study.
It is permitted and recommended to take vitamins (not containing iron), folic acid, and ascorbic acid by medical prescription, as well as adhere to a diet with products rich in iron.
It is not recommended to take non-steroidal anti-inflammatory drugs groundlessly, including selective inhibitors of cyclo-oxygenase, but these drug products may be prescribed by the Investigator due to medical indications.

6.5 Prohibited concomitant therapy

During the study the enrolled patients must not administer drugs:
• indicated for the treatment of iron deficiency anemia:

Pharmaceutical groups	**Active ingredient (INN)**
Dietary supplements, vitamin and mineral complexes	
Dietary supplements macro and micronutrients	
Vitamins and vitamin-like products	Vitamin C
	Pyridoxine
	Riboflavin
Vitamins and vitamin-like products in combinations	Multivitamins + Minerals
Macro and micronutrients	Ferrous protein succinylate
Macro and micronutrients in combinations	Hematogen
	Ferrous Sulfate + Serine + Folic Acid
	Multivitamins + Minerals
Hemopoiesis stimulants	Ferrous (III) hydroxide polymaltozate
	Ferrous (III) hydroxide sucrose complex
	Ferrous gluconate

	Ferrous carboxymaltose t
	Ferrous protein succinylate
	Ferrous sulfate
	Ferrous fumarate
	Ferrous chloride
	Cyanocobalamin
Hemopoiesis stimulants in combinations	Ferrous (III) hydroxide polymaltozate + Folic acid
	Ferrous sulphate + folic acid + Cyanocobalamin
	Ferrous sulfate + folic acid
	Ferrous fumarate + folic acid

as well as any other drugs that affect blood formation (for example, cytostatics, interferons, chloramphenicol, streptomycin).
• Other drugs stimulating hematopoiesis (epoetins, anabolic steroids, etc.)
- Drugs that slow iron absorption (antacids, calcium supplements) 2 hours before administration of the investigational products. It is not recommended to drink strong tea, coffee 2 hours before medications administration.

During the study, patients should not undergo any physiotherapeutic procedures and other procedures potentially affecting erythropoiesis (for example, Buteyko breathing technique).

7 Assessment of efficacy

7.1 Methods and periods for registration of efficacy parameters

The efficacy of the study therapy is based on laboratory and clinical data.
Laboratory parameters for effectiveness evaluation will be collected at screening visits (Visit 0), Visit 2, Visit 3 and the final visit (Visit 4):
- Complete blood count (hemoglobin);
- Iron metabolism indices (ferritin, transferrin, transferrin saturation percentage, serum iron).

7.2 Efficacy endpoints

Primary efficacy endpoint*:*
- Changes in hemoglobin level (g/L) in 12 weeks of iron deficiency therapy (at the final visit) as compared to baseline (determined at the screening visit) in the study groups.

Secondary efficacy endpoints:
- Absolute values and changes in hemoglobin level (g/L) in 4, 8 and 12 weeks of therapy (determined at the final visit) between the study groups.
- Changes in iron metabolism parameters mean values (ferritin, transferrin, transferrin saturation percentage, serum iron) within the therapy period (from the screening to the final visit) in the study groups.
- Responder rate (%), determined as an increase in hemoglobin level by 20 g/L and more after 12 weeks of treatment (from screening till the final visit) in the study groups.

8 Safety evaluation

8.1 Methods and periods for registration of safety parameters

Safety evaluation of the study therapy will be performed throughout the study and will be based on detection of any adverse events occurring during the study. The following will be performed:

- Physical examination, including measurement of blood pressure and heart rate, and gastrointestinal disorders identification (at the screening, during the second visit and the final visit).
- Complete blood count (at screening and in 4, 8 and 12 weeks of therapy),
- Blood biochemistry (at screening and after 12 weeks of treatment),
- Clinical urinalysis, pregnancy test (at screening and in 12 weeks).
- Registration of concomitant therapy (during each visit).

8.2 Safety parameters

Procedure for primary safety parameters assessment (including physical examination, laboratory and instrumental examinations) is detailed in the section 4.2. Safety evaluation will be performed throughout the study (see the planned procedures for visits in the section 4.2.9).

8.3 Requirements for reporting, registration, and reporting of adverse events and intercurrent diseases

8.3.1 Adverse Events / Serious Adverse Events Definitions

Adverse Event (AE) — any untoward medical occurrence in a patient or clinical study subject after administration of a medicinal product which does not necessarily have a causal relationship with this treatment.
An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
Serious Adverse Event (SAE) is any unfavorable medical occurrence that at any dose:

- results in death
- Is life threatening;
- Requires hospitalization or its extension;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant: medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical occurrences that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition. These should also usually be considered serious.

The characteristics/consequences should be considered at the time of the event. For example, the term "life-threatening adverse event" refers to a medical occurrence in which the subject is at risk

of death at the time of the adverse event; it does not refer to a medical occurrence which hypothetically might have caused death if it were more severe.
(Serious) Adverse Reaction ((S)AR) is any (S)AE for which a causal relationship with a medicinal product is regarded by the Investigator or Sponsor as plausible (see Section 8.8.3 below).
(Serious) unexpected adverse reaction is defined as (serious) adverse reaction, the character or severity of which does not correspond to the safety information of the drug (for example, Summary of product characteristics [SPC], drug product certified pharmacopoeial description, Investigator's Brochure) The term "severity" is used here to describe the intensity of a specific adverse event. This has to be distinguished from the term 'serious'. Reports which add significant information on the specificity, increase of occurrence, or severity of a known, already documented serious adverse reaction constitute unexpected events.

Information on common known side effects of the Investigational Medicinal Product (IMP) can be found in the Reference Safety Information (e.g. SPC, Product Monograph, Investigator Brochure). Otherwise, it will be communicated in the form of Investigator Notification. This information will be included in the Patient Information Sheet and should be discussed with patients.

For further details, please refer to the Quick Reference Guide for completing the Serious Adverse Event Report Form.

8.3.2 Severity of Adverse Events

The term "severity" is used here to describe the intensity of a specific event. This has to be distinguished from the term 'serious'. During the trial, the Investigator should define any emerging AEs and rate their severity as follows:

Mild	an AE that is usually of transient nature and, as a rule, does not limit daily activity.
Moderate	Sufficiently discomforting to interfere with normal activities
Severe	prevents routine daily activity.

8.3.3 Relation to the IMP

The Investigator should evaluate all AEs considering all accessible data, at any time new information becomes available. The definition of IMP includes the test product and the comparator drug or placebo administered during any phase of the trial.

The Investigator should assess whether, in his/her expert opinion, the relationship of the AE to the drug is suspected based on the following considerations:

Suspected:	The temporal relationship of the clinical event and the study drug administration is possible, while other drug products, therapeutic interventions or an underlying condition do not provide a sufficient explanation for the observed event.
Not suspected:	The temporal relationship of the clinical event and the study drug administration is unlikely,

	or other drug products, therapeutic interventions or an underlying condition provide a sufficient explanation for the observed event.

Causal relationship assessments are critical and must be provided for each individual AE in relation to each IMP, non-investigational medicinal product (NIMP) or other concomitant medicinal product, if applicable. Missing causality assessments will be handled as suspected to IMP by the sponsor.
AEs with a suspected relationship to NIMP or other concomitant medication (for both Sandoz and non-Sandoz products), even if non-serious, need to be reported by the investigator to the respective Country Organization Patient Safety Team (see section 8.3.5 for contact details).

8.3.4 Adverse Events Documentation

Any AE (non-serious and serious) occurring after the subject has provided study-specific informed consent and until the last study visit of the subject, has to be recorded on the AE pages of the Case Report Form (CRF).

The data on AEs should be collected by nonintrusive questioning of the subject at each visit during the study. AEs also may be identified when they are volunteered by the subject during or between visits or during physical examination, laboratory test or other assessments. All AEs should be given appropriate medical care. Treatment may include one or more of the following actions: no action is taken (i. e. further observation only); IMP dosage is adjusted/temporarily interrupted; IMP is permanently discontinued due to this AE; concomitant medicinal product is given; non-drug therapy is given; patient is hospitalized/patient's hospitalization is prolonged. The treatment of the AE should be documented in the CRF. In addition, the actions taken concerning the IMP should be documented and assigned to one of the following categories: "no changes", "discontinuation", "dose reduction", "dose increase", "interruption", "unknown", and "not applicable". Concomitant medicinal product, other types of treatment, or changes in the administration of the IMP should be specified and documented.
Medical conditions/diseases present before starting IMP are only considered to be AEs in the case of their worsening after the patient enrolment. Abnormal laboratory findings or other test results constitute AEs only if they induce clinical signs or symptoms, are considered to be clinically significant, or require therapy.

Once an AE is identified, the investigator should follow-up as specified below. Each time, the outcome should be documented and assigned to one of the following categories: "not resolved/unchanged", "condition deteriorating", "recovered/resolved", "improving/recovering", "recovered/resolved with sequelae", "fatal", or "unknown". The assessment of the AE should be performed during each planned visit (or more frequently, if necessary). The Investigator should document in the CRF any changes in seriousness, severity, the suspected relationship with the IMP, the interventions required to treat the AE, as well as its outcome.

<u>Adverse events occurring between informed consent and the last visit</u>
The Investigator should continue observations for all AEs which occurred in the period between the study-specific informed consent signing and the last visit of the subject, at which the outcome assessment is documented in the CRF.

<u>Serious adverse events still ongoing at the time of the last visit</u>

For any SAEs still ongoing at the time of the last visit, the Investigator should continue observations until the SAE has resolved, stabilized or is judged permanent in the case of SAEs considered to be related to IMP (SARs), or for up to 30 days after the last visit of the subject in the case of non-related SAEs. The Investigator should send SAE follow-up reports to recipients as per Section 8.8.5, SAE reporting, presented below.

Serious adverse events occurring after the last visit
SAEs registered after the last visit should be reported to the Sponsor only if they are evaluated by the study physician as related to drug administration. The Investigator should send SAR follow-up reports to recipients as per Section 8.8.5, SAE reporting, presented below.

8.3.5 SAE Reporting

It is vital that the investigator reports any SAEs, or updates to previously reported SAEs **immediately**, i.e. not later than 24 hours after the information has been received, even if the investigator does not consider the AE to be drug-related.
To report on a SAE the Investigator should send a Serious Adverse Event Report Form (a Novartis document) as an initial or follow-up report, via fax or e-mail to the relevant Country Organization Patient Safety Team with a copy to the accountable study manager (Sponsor/CRO) to the addresses given in the table below.

The Investigator should also send all updates/new information on a new SAE Report Form as a follow-up data to previously reported SAE. The follow-up data should describe whether the adverse event resolved or is ongoing, if a diagnosis is available, if (and how) it was treated, and whether the subject is continuing or was withdrawn from the trial.
Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up information to the original episode, regardless of when the adverse event occurs.
Any new SAE (that is considered completely independent of a previously reported SAE) should be reported as a new and separate initial SAE report.
Any queries from the Country Organization Patient Safety Team, Contract Research Organization (CRO), or Sponsor regarding SAE reports should be answered by the Investigator within 24 hours.
For further information see Brief Reference Guide for filling out of the Serious Adverse Event Report Form

The Investigator should retain SAE reports delivery confirmations to all recipients in the Investigator File.

Addresses for SAE reporting

[redacted] **Novartis Pharma**
Full name: [redacted]
Address: [redacted]
Phone: [redacted]
Mobile: [redacted] **(24h)**
Fax: [redacted]
E-mail: [redacted]

[redacted] Sandoz (a Novartis division):

Full name: [redacted]
Address: [redacted]
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

Contact persons in charge for handling questions:

Sandoz: Full name: Address: Phone: Mobile: Fax: E-mail:	CRO: Full name: Address: Phone: Mobile: Fax: E-mail:

Investigator Notification and 6 monthly line listings

If a SADR is not listed in the Reference Safety Information (e.g. SmPC, Product Monograph, Investigator's Brochure), the sponsor may urgently require further information from the investigator for Health Authority reporting.

The Sponsor may, where applicable, forward Investigator Notifications and biannual tabulated listings of Suspected Unexpected Serious Adverse Reactions (SUSARs) to all Investigators taking part in any clinical trial covering the same IMP.

The submission of these Investigator Notifications and biannual listings, if applicable, to the local Institutional Review Board/Ethics committee is the responsibility of the Investigator or the CRO as stipulated in the study contract. The submission of Investigator Notifications and 6-monthly line listings to national Ethics Committee is the responsibility of the CRO, if applicable.

Health authority reporting

The Sponsor submits all reportable cases within the required timelines to all concerned authorities.

8.3.6 Pregnancy

All cases of pregnancy among subjects during the study must be **immediately (within 24 hours)** reported by the Investigator to the Country Organization Patient Safety Team, with a copy sent to the study manager (Sponsor / CRO), as described in Section 8.3.5. Pregnancies are only reported from the date of the first IMP dose administration.

The Investigator should immediately discontinue the subject from the study, and should follow-up each case of pregnancy, and report its outcome, including spontaneous or voluntary termination, and submit detailed data on birth, any birth defects, congenital abnormalities, or maternal and/or newborn complications.

If it becomes known to the Investigator that a male subject has impregnated his/her partner between the first dosing and the final visit (paternal exposure), this pregnancy should also be reported.

Pregnancies are reported on the Case Report of Pregnancy Exposure Form (a Novartis document). Information on pregnancy follow-up monitoring of is reported using the same form. It should include an assessment of the relatedness of any untoward pregnancy outcome to the IMP.
Any SAE during pregnancy of a subject must be reported on the SAE Report Form and reported as described in Section 8.3.5

The Investigator must store in investigator's file information about the delivery of forms to all recipients of messages on the Form.
For further information see Brief Guidance for completing the form of a report on the effects of the drug during pregnancy (a Novartis document).

8.3.7 Complaints related to IMP/medical device

In the case of claims in respect of quality of IMP/medical device, Investigator shall inform the Sponsor within 24 hours after receiving the information on such a claim in accordance with the instructions given in Appendix 4.3.2. Any AEs related to quality claims must be documented and reported by the Investigator in addition as described in Sections 8.3.4and 8.3.5.

8.3.8 Special cases

Special cases can be serious or non-serious (see table below). They should be reported as cases of AEs, as described in Sections 8.3.4and 8.3.5, even if no (other) AEs are associated with them.

Special case	**The Investigator notifies the Country Organization Patient Safety Team within 24 hours**
Drug exposure during breastfeeding	X
Intentional Overdose by patient (including suicide attempts, suicide attempt is always serious)	Only if associated with SAE
Interaction with other medicinal products	Only if associated with SAE
Withdrawal syndrome/reaction	Only if associated with SAE
Drug dependence, misuse, abuse or addiction (always treated as serious event)	X
Suspected transmission of infectious agents (always serious)	X
Death (including cases in the absence of other adverse events, is always treated as a serious event)	X

8.3.9 Reconciliation

Reconciliation between the safety database of the sponsor and the clinical database at the CRO will be done periodically / at the end of the study as described in a reconciliation plan / by comparing line listings from the safety database with the data in the clinical database.

Data managers will study the CRF/clinical database for possible cases that have not been reported. For any case to be reported, the following parameters must exactly match in the clinical database and the safety database: number of study, number of study site, number of participant/patient number, randomization number, investigational product, seriousness, date of death (if applicable), and Investigator assessment of causality. All other parameters should be coordinated from a medical point of view and believably consistent with each other. For any case to be reported and evaluated as being presumed to be associated with treatment, or for other AEs of particular interest (if applicable), more detailed agreement should be made, including also the dates of treatment, outcomes, history, and concomitant therapy.

8.3.10 Investigator Training

By his/her signature of the study protocol, the principal investigator certifies that he/she has been trained in the Sponsor AE/SAE and pregnancy reporting obligations by the CRO as defined in the study protocol.

9 Statistical methods description

9.1 Analysis methods description

Statistical analysis will be performed using R Project specialized software.
All continuous (quantitative) indicators will be submitted in the form of the following descriptive statistics parameters:

- Number of observations,
- arithmetic mean,
- 95 % confidence interval (CI) for the average,
- standard deviation,
- median,
- interquartile range,
- minimum,
- maximum.

All the ordinal, categorical and qualitative indicators will be submitted in the form of the following descriptive statistics parameters:

- absolute frequency (observations number),
- relative frequency (expressed as percent),
- 95% CI for proportion.

All the performed types of statistical analysis will be identical in the study product Ferrum Lek® group and in the comparator MALTOFER® group.
The concomitant diseases and AEs will be coded using MedDRA terms.
This section contains a brief description of the planned analysis. Full analysis will be presented in the statistical anaysis plan.

9.1.1 Demographic and other baseline data (group comparability for the analysis)

All the data obtained in the groups before the study therapy initiation (demographic, laboratory, instrumental, and physical examinations data, vital signs, etc.) will be compared between the groups to determine the groups comparability for analysis.
The Fisher's exact test and χ^2 (chi-square) will be used to compare the qualitative and serial data, while the t-test or the Mann–Whitney test will be used for the quantitative data (depending on the quantitative data distribution).
To assess the normality of the distribution, the Shapiro-Wilk Test will be applied.
In the event that any of the initial data reveal the incomparability of the study groups (statistically significant differences in demographic and other initial data between the groups), the analysis of the efficacy and safety parameters will be additionally performed, together with the primary planned analysis, using multi-factor statistics (ANOVA, ANCOVA or logistic regression analysis depending on the type of the studied parameter), as adjusted for the initial indicator(s) that vary between the groups.

9.1.2 Analysis of the primary efficacy endpoint

Primary efficacy endpoint:

- Changes in blood haemoglobin level (g/L) after 12-weeks of iron-deficiency anaemia treatment (during the final visit), as compared with the baseline value (the screening visit) between the study groups.

Analysis of covariance (ANCOVA) will be used as the main method of assessment of the primary endpoint, with baseline haemoglobin value being a covariate and four fixed factors (treatment group, gender, weight and baseline Hb level (80–94 g/L vs 95–110 g/L). Unrestricted least significant differences (LSD) method will be applied to the ANCOVA results with the calculation of least-square (LS) means with 95% CI for LS means for the difference between the study groups. Non-inferiority will be confirmed if the upper limit of the two-sided 95% confidence interval for LS mean will not exceed the pre-defined non-inferiority margin of 5 g/L.

9.1.3 Analysis of the secondary effecacy endpoints

Secondary efficacy endpoints:
All secondary efficacy analysis will be considered explorative, therefore, no multiplicity correction will be done for any tests, and no formal power calculation will be provided for the test results.

- Absolute values and changes (compared to baseline) of blood hemoglobin level (g/L) after 12 weeks of treatment (during the final visit) between the study groups.

Values at individual time points and values of change between the individual time points will be compared using the t-test or Mann-Whitney test depending on the normality of the distribution (which will be tested using Shapiro-Wilk test).
Additionally, it is planned to use ANCOVA for analysis of changes in the assessed parameters over time where group will be a fixed factor, screening hemoglobin level will be a covariate , and hemoglobin level at Week 12 will be a response variable.

- Changes in average values of iron metabolism parameters (ferritin, transferrin, percent transferrin saturation, serum iron) during the treatment period (from screening till the final visit) in the study groups.

Values at individual time points (baseline and Week 12) and values of change between the individual time points (baseline and Week 12) will be compared using the t-test or Mann-Whitney test depending on the normality of the distribution (which will be tested using Shapiro-Wilk test).

- The percentage (%) of subjects with response to the therapy, determined as an increase in hemoglobin level by 20 g/L and more after 12-weeks of treatment (from screening till the final visit) in the study groups.

Comparison between the two groups will be performed using the Fisher's exact test or chi-square test depending on the number of expected observations per cell (<5 or ≥5). Two-sided 95% confidence interval will be calculated using exact Clopper-Pearson method.
These changes will be compared between the groups using the t-test or Mann-Whitney test depending on the normality of the distribution (which will be tested using Shapiro-Wilk test).

9.1.4 Safety parameteres analysis

Safety evaluation will include:
1) determination of the total number, incidence and severity of the folowing:
   I. adverse events (AEs), regardless of their relationship to the treatment;
   II. AEs associated or potentially associated with the drug;
   III. AEs leading to discontinuation.
2) Gastrointestinal disorders (determined by means of a standardized MedDRA query for the corresponding symptoms), regarded by the Investigator as related to the study therapy, will be analyzed separately.
Methods of descriptive statistics will be used to represent the results. A comparison of the incidence of new AE cases in the study groups will be performed using the Fisher's exact test or

the chi-square test, depending on the number of proposed observations in one cell (<5 or ≥5), and a comparison of the severity (and the possible causality between the study drug and AE) will be conducted using the Cochran—Armitage test for linear trends for ordered categorical data.
3) Changes in the results of laboratory studies (conducted in the central laboratory) over time and the incidence of abnormal results of studies (based on the reference values of the central laboratory) will be summarized by group, and a comparison will be made between the groups using appropriate tests for quantitative and qualitative data (see below).
4) Changes in the vital signs over time and the incidence of pathological abnormalities revealed during physical examination will be summarized by group, and a comparison will be made between the groups using appropriate tests for quantitative and qualitative data (see below).
Comparison of quantitative data will be conducted using the t-test or the Mann—Whitney test, depending on the distribution normality (which will be estimated using the Shapiro—Wilk test).
Qualitative data will be compared using the exact Fisher's exact test or the chi-square test, depending on the number of proposed observations in one cell (<5 or ≥5).

9.2 Interim analysis

One formal Interim analysis to assess the primary and secondary endpoints of efficacy is planned when the population of patients with available primary efficacy endpoint data is at least 240 patients (120 patients in each group treatment: the main group is Ferrum Lek® (iron (III) hydroxide polymaltosate) and the reference group MALTOFER® (iron (III) hydroxide polymaltosate). Safety and Efficacy will be assessed for all patients who received at least one dose of the randomized treatment at the time of the interim analysis. Based on the results of the interim analysis of the primary and secondary statistical endpoints of the efficacy and safety of the study Sponsor may decide to stop the study if study endpoints have been met as per protocol based on the results of interim analyses or sample size might be recalculated using results of interim analyses.

9.3 Target number of the clinical trial subjects with the sample size justification

Calculation of the sample size is based on published works focusing on iron (III) hydroxide polymaltosate efficacy:

7) Maltofer Product Information leaflet (http://www.aspenpharma.com.au/product_info/pi/PI_Maltofer.pdf)

8) Santiago P. Ferrous versus ferric oral iron formulations for the treatment of iron deficiency: a clinical overview. Scientific World Journal. 2012;2012:846824.

9) Geisser P. Safety and efficacy of iron(III)-hydroxide polymaltose complex / a review of over 25 years' experience. Arzneimittelforschung. 2007;57(6A):439-52;

10) Toblli JE, Brignoli R. Iron(III)-hydroxide polymaltose complex in iron deficiency anemia / review and meta-analysis. Arzneimittelforschung. 2007;57(6A):431-8);

11) Reinisch W, Staun M, Tandon RK, Altorjay I, Thillainayagam AV, Gratzer C, Nijhawan S, Thomsen LL. A randomized, open-label, non-inferiority study of intravenous iron isomaltoside 1,000 (Monofer) compared with oral iron for treatment of anemia in IBD (PROCEED). Am J Gastroenterol. 2013 Dec;108(12):1877-88.

12) Zaim M, Piselli L, Fioravanti P, Kanony-Truc C. Efficacy and tolerability of a prolonged release ferrous sulphate formulation in iron deficiency anaemia: a non-inferiority controlled trial. Eur J Nutr. 2012 Mar;51(2):221-9.

Based on the data of these publications, during 12-weeks of oral administration of iron (III) hydroxide polymaltosate the hemoglobin level can increase on average by 8-15 g/L with a standard deviation up to 15 g/L. As similar efficacy is expected in both groups, zero difference in primary efficacy parameter will be used for calculation of sample size. Maximum value of standard deviation was used – 15 g/L to take a maximum variability of the primary endpoint variable into account for properly powered study.
The value of 5 g/L was selected as the non-inferior efficacy limit (in accordance with the limit stated and justified in the above mentioned works.
Thus, the following assumptions were proposed for calculations:

10) Changes in blood hemoglobin level (g/L) after 12-weeks of iron-deficiency anemia treatment (during the final visit), as compared with the baseline value (the screening visit 0) are expected to be similar for the investigational and reference drug groups (i.e. expected difference between the two groups is set to zero).

11) The pooled standard deviation for the changes in hemoglobin level was 15 g/L.

12) Non-inferiority margin is 5 g/L.

13) The significance level (two-sided, in accordance with FDA recommendations) is 95%, which corresponds to the type I one-sided error 0.05.

14) Power of the study is 80%, which corresponds to the type II error 0.20.

15) Statistical hypotheses – evidence of a non-inferior efficacy:

$$H_0: \mu_A - \mu_B \leq 5.0$$
$$H_1: \mu_A - \mu_B > 5.0$$

16) The ratio between the study and control group sizes is 1:1

17) Interim analysis will be performed at 70% of the total sample size

18) O'Brien-Fleming alpha-spending function will be used with 80% power for efficacy boundary that will correspond to the following critical values for t-test: 2,628 for interim analysis (corresponds to p=0,005) and 1,976 for final analysis (corresponds to p= 0,0245);

Calculation was performed using the clinfun package of Microsoft R Open software (available at https://mran.microsoft.com – Microsoft R Application network), that uses the group sequential design approach for the calculation of fixed sample sizes for the interim and final analysis. According to the output from the clinfun package, 143 subjects per group (286 subjects in total) should complete the study in order to confirm non-inferiority. 200 (100 per group) subjects should have evaluable data to perform interim analysis. Taking 15% withdrawal rate into account, one need to randomize 336 subjects (168 subjects per group). Taking 30% screening failure rate, up to 480 subjects should be screened to achieve target randomization.

9.4 Applied significance level of the clinical trial

All the statistical tests within this study will be performed at 95% confidence level (threshold value p to confirm stastistical significance is less 0.05). The two-sided statistical criteria will be used for all other studied parameters.

9.5 Clinical study termination criteria

The trial may be terminated under conditions described in Section 4.8of this protocol.

9.6 Accounting for missed, unanalyzable, and questionable data

During the monitoring visits to the trial sites, the clinical trial specialists (monitors) authorized by the Sponsor will analyze eCRFs for the data completeness. In the absence of data on CRFs and the availability of relevant information in the primary documentation, questions for investigators and regulations to address inconsistencies will be formulated.
At the database check, the expert in statistics authorized by the Sponsor, and the data control and processing managers will analyze the trial results for questionable, missed, and unanalyzable data, which might be the basis for the questions for investigators.
If possible, the investigators will eliminate the errors identified in eCRFs and inform the Principal Investigator and authorized representatives of the Sponsor hereof. In case the identified data errors cannot be eliminated after the completion of the patients participation in the trial, the analysis of the resulting parameter sensitivity to questionable data will be conducted in the statistical analysis.
Information about the missed, questionable, and unanalyzable data will be summarized in the final clinical trial report.
A method of filling in the missing data based on maximum likelihood estimation, MLE for the primary end-point will be applied if needed[64].
Analysis of the rest of endpoint types and other parameters will be performed only on the basis of the actually available information without filling in the missing data in view of short duration of the clinical trial.

9.7 Procedures for reporting any deviations from the original statistical plan

All deviations from an initial statistical plan must be described and justified in a protocol amendment and/or a final trial report (in the latter case, a statistical analysis plan developed before the start of a final statistical analysis must contain a list of those deviations with justification for the reasons thereof).

9.8 Procedures for clinical trial participants selection with a view to analysis

The following data sets will be used for analysis:

- The intention-to-treat (ITT) population: all randomized subjects who were administered at least one dose of the investigational product or comparator and who have hemoglobin level data both before and after treatment with the investigational product and the reference product.

In accordance with principles of ICH E9 guidelines, the ITT population will be considered to be a population for statistical analysis.

- The per protocol (PP) population: all randomized subjects who have completed participation in the trial in accordance with the protocol (have completed the prescribed period of treatment and follow-up without significant deviations from the protocol).
- The safety population (safety): All randomized subjects who received at least one dose of the study drug/comparator drug and completed at least one safety parameters evaluation visit. Unlike the ITT population, the safety population will be analyzed depending on actually received treatment rather than not only prescribed (in the case of difference detection between the prescribed and received therapies).

10 Description of arrangements for quality control and quality assurance

10.1 Trial monitoring and quality control

Regular visits by a clinical trial specialist (Monitor), on request of the Sponsor and according to SOPs, before initiation, throughout, and upon completion of the trial contribute to a successful trial conduct and accurate data collection, timely detection of errors, proper documenting of the trial process, protection of the subjects' rights, and consistence with the principles of ICH GCP, international and Russian laws.

Routine trial monitoring includes:

- Confirmation for the proper conduct and documenting of the informed consent receipt, screening, and inclusion of the subjects in the trial.
- Verification of the data in CRF and primary medical documents.
- Confirmation for documenting and timely reporting of AEs during the trial;
- Confirmation for compliance of the study site personnel with the requirements for diagnostic and therapeutic procedures stipulated by the protocol;
- Confirmation for documenting the shipments, storage, distribution, and utilization of the study product/comparator drug and trial materials;
- Confirmation for competence of the study site personnel and external laboratories needed for the trial conduct.
- Confirmation for compliance of the diagnostic and laboratory equipment with the safe use requirements during the trial.
- Confirmation for cooperation of the Investigator with the local Ethics Committee regarding the trial safety and introduction of the protocol amendments agreed upon by the Sponsor.

Quality control of the trial results by the Sponsor's authorized persons or representatives who keep a trial electronic database, reveal for any inconsistence, data imputed by error, or missing data during the cross check of all eCRFs. In the case of any questions or necessity of clarification, a special form (a data clarification request) should be forwarded to the Investigator and satisfied in writing during 7 days since the receipt thereof.

In accordance with the requirements of the legislation, the Sponsor or authorized federal authorities have the right to inspect (audit) the logistical support and documentation on the trial. The Investigator is obliged to provide direct access to documents and all relevant information to the authorized persons for the audit or inspection.

10.2 Amendments to the protocol

The investigators' signatures on a signature list of the protocol mean a written consent to perform a trial in accordance with this protocol. During the trial, the trial materials may be amended and appended. Such changes and additions are considered to be amendments.

A protocol amendment is a written description of changes or formal clarification of the clinical trial protocol wordings. The amendments may be significant and insignificant. Any protocol amendment, before making effective, must be approved according to the established procedure and SOPs of the Sponsor, than approved by regulatory authorities and signed by the Investigator.

Order of the Ministry of Health of Russia On Approval of a Review Procedure of a Message on Necessity to Introduce Amendments to a Protocol of the Clinical Trial of the Drug No. 775 of August 31, 2010 contains a list of significant/insignificant amendments and a procedure of submission thereof for the expert evaluation.

The protocol amendments are significant if they may influence objectives, organization, methods of the trial, statistical methods of data processing, and patients' safety measures during the trial.
The protocol amendments are insignificant if they may not influence the objectives, organization, methods of the trial, statistical methods of data processing, and measures for patients' safety during the trial.
In the case of necessity to introduce changes into this protocol, the Investigator shall submit a message on necessity to introduce amendments to the clinical trial protocol to the Ministry of Health of Russian Federation. The Ministry of Health of Russia shall consider the submitted documents and decide on approval or refusal to the introduction of changes. The protocol amendments must be stored together with the initial protocol version. A title page of the protocol must contain a number and date of the amendments.

10.3 Protocol deviations

Protocol deviation is an unintentional deviation from the approved protocol.
Serious protocol deviation is a deviation, which may, in the opinion of the Investigator or a person assigned by the Investigator, result in withdrawal of the subject from the study or exclusion of his data from bioanalytical and/or statistical parts of the study. The deviations that were no classified as serious shall be considered minor protocol deviations.
Serious deviations from the Protocol should be reported as soon as possible to the Sponsor by the Site staff and/or CRO and Monitor (if he/she is in the Site). The Sponsor may propose to reclassify the protocol deviation (minor into serious and vice versa) based on the conducted evaluation. In such a case, the classification performed by the Sponsor shall prevail and must be communication to CRO together with written rationale.
The Sponsor must be informed of minor protocol deviations within 10 business days but before the start of the next study period or before the start of the bioanalytical/statistical phase.
An exception is represented with minor protocol deviations, which can be included in the draft report on the trial:

- Logistic deviations (deviation in time from the planned blood sampling schedule; follow-up visit which were outside the time limits set up in the Protocol due to a participant's plans/schedule, etc);
- Administrative deviations (e.g. change of the name).

If less than planned number of volunteers is enrolled, the CRO should contact the Sponsor, who may approve using smaller than planned number of volunteers. It is not considered to be a deviation from the Protocol.
Notification and reporting on deviations from the Protocol shall be submitted to the Regulatory authorities and relevant Ethical Committees in compliance with the applicable requirements/instructions/laws.
Procedure for documentation of Protocol deviations
The Investigator or a person assigned by the Investigator must document and explain any deviation from the approved Protocol. Notification of the Sponsor on a protocol deviation can be verbal in exceptional cases (if immediate action/notification is required), and must be followed with written report (e.g. via e-mail; during updates on the study period process). All Protocol deviations must be described in the final report on the study.

11 Description of ethical aspects of the clinical trial

11.1 General provisions

The trial will be performed in accordance with the principles and requirements set forth in the documents:

- Declaration of Helsinki, World Medical Association (adopted at the 18th Assembly of the World Medical Association in Helsinki in June 1964, the latest revision adopted by the 64th Assembly in Fortaleza in October 2013),
- ICH E6 Harmonized Tripartite Guidelines for Good Clinical Practice dated 09.11.2016
- Guideline for Good Clinical Practice of the Eurasian Economic Union (adopted through the decision No. 79 of the Council of Eurasian Economic Commission dated November 3, 2016);
- Federal law dated April 12, 2010 № 61-FZ "On Circulation of Medicines" (as amended);
- Order No. 200n dated April 01, 2016 of the Ministry of Health of the Russian Federation "On Approval of Rules for Good Clinical Practice";
- The national standard of the RF GOST R 52379-2005 "Good Clinical Practice".

The study will be initiated in all research centers only after obtaining a written permission to conduct research and approval of the Ethics Committee of the Ministry of Health of the Russian Federation and Local Ethics Committees and getting the signatures of each party participating in the study in the Clinical Trial Protocol.
Investigators will be familiarized with the study materials in time prior to the study initiation. Investigators' qualification will meet the requirements essential for conducting high-quality clinical trials.
Selection of prospective study participants is carried out on a voluntary basis.
Ethics Committee
Clinical trial materials (including CRF, materials provided to the patient) will be approved by the Ethics Committee of the Ministry of Health of the RF and Local Ethics Committees prior to the trial start. All the amendments to the Protocol and updated versions of the Patient Information Leaflet will be submitted to the Ethics Committee for approval.
The clinical trial will be performed in accordance with the Protocol approved by The Ethics Council.

11.2 The informed consent receipt procedure

Before the inclusion, a subject is provided with written information and verbal clarification on objectives, goals, and methods of the trial, as well as expected benefit and possible risks associated with the participation in the trial. Besides, the subjects must be informed on voluntary nature of the participation in the trial and their rights to refuse to participate at any moment without influence on quality of the provided medical care. While a subject is not obliged to report his/her reasons to terminate his/her participation in the trial, the Investigator should try to find out the reasons without violating the subject's rights. The subject's consent must be obtained before initiation of any of the trial procedures.
Processing of the data gathered during the trial is performed with the maintenance of confidentiality of personal data. The subjects should be made aware of the goals of the planned computed data processing and publication terms (e.g., on medical conferences, in magazine articles, and other publicly open sources), when the data is presented in summary not allowing for the subjects identification.

Subjects should be made aware that the authorized representatives of healthcare authorities and of the Sponsor will have access to their confidential health information for the purpose of monitoring, inspection, and audit. The subjects must receive guarantees of the strict confidentiality and non-disclosure of all personally identifiable information.
Patient Information Sheet with the informed consent form should be filled in in duplicates, signed, and dated by the subject and Investigator in person. One copy of the signed Patient Information Sheet with the Informed Consent Form should be kept in the Investigator's file, the other copy should be given to the subject.

11.3 Confidentiality and identification of the trial subjects

Personally identifiable information will be kept confidential in observance of the right to privacy and confidentiality protection according to the applicable laws. Personally identifiable information will be kept confidential and may be disclosed only to the extent required by law. On publication of the trial results, confidentiality of the personally identifiable information will be maintained.

11.4 Enrollment of subjects from the vulnerable and special populations

The inclusion/non-inclusion criteria do not envisage for participation of the subjects from the vulnerable populations.
Special populations in this trial include women with childbearing potential who, according to the inclusion/non-inclusion criteria, may participate only upon their consent to use effective contraception during the trial which is stated in the patient information sheet and confirmed by signing of the Informed Consent Form.

12 Data processing and record keeping

All the trial relevant records and documents of the trial site, including those contained in the Investigator's file (eCRFs, Informed Consent Forms, logbooks, subject accounting sheets, etc.), as well as primary medical documentation of the subjects must be stored for 25 years after the trial completion. The study Sponsor must control storage and availability of all the study site materials during a life cycle of the investigational drug. Archival data may be stored as photocopies, as well as on the optical or electronic data storage devices. The Principal Investigator must inform the Sponsor immediately on the unintentional damage/destruction as well as change of the storage place of the archival data from the clinical trial. A targeted destruction of the archival data may be performed only upon a written permission of the Sponsor.
As far as the subjects perform visits to the trial sites and the Investigator fills in the eCRFs, the authorized monitors of the Sponsor will verify the data in eCRFs and primary documents. In the case of any questions arising during the eCRFs data check on part of a data control manager and/or a biostatistics specialist, all the clarifications and changes will be documented by creating a special form (a data clarification request).
The trial must be conducted in accordance with the protocol and applicable Sponsor's SOPs. If the protocol needs to be changed, one should follow a procedure described in Section 10.2 of this protocol.
The Investigator must fill in all the primary medical documents and eCRFs for all the included subjects.

The Investigator is responsible for the complete and accurate filling of the eCRFs. All the data entered in eCRFs must be also recorded in subject's medical records in printed form or in the form of records made by the Investigator or another authorized person of the trial site.
The eCRFs, in accordance with the primary documents, should contain all significant information on the subject's participation in the trial. Besides, eCRFs should contain data on the trial completion by the subject. The eCRFs must be filled in not less than 5 days after the subject's visit to the trial site.
confidential. The Investigator may disclose the trial information to the parties not directly involved in the trial only upon the Sponsor's permission.

All data provided for the evaluation will be integrated into the final clinical study report which will describe the methodology for conducting statistical analysis, as well as clinical conclusions regarding efficacy and safety.
The CS report will be prepared by the CRO in cooperation with the Sponsor. The principal investigator of each study site will receive a copy of the Report for review and subsequent signing. The structure and content of the Report will generally comply with the requirements of the ICH E3 guidelines, as well as applicable local regulatory requirements.
If recalculation of the sample size using the results of the interim analysis is necessary, Report on the interim analysis results will be generated, it will be submitted to the regulatory authority of the Russian Federation with a view to obtaining permission to continue the clinical study involving an increased number of patients.

13 Direct access to primary data/documents

Primary data represent entire information contained in original records and certified copies regarding clinical data, observations, and other measures within the trial scope and which is required for reconstruction and evaluation of the trial. The investigator must cooperate for the purposes of monitoring, audit(s), expert evaluation by the Ethics Committee and regulatory authorities and provide the access to the primary data/records.
The primary data must be stored in a proper quality throughout the time stipulated for by the local and international laws, as well as written contracts with the Sponsor company. For each subject included, the Investigator must note in the primary records the fact of such participation as well as the following data: an individual identification code, personal data (full name, address), dates of the drug intake, vital signs, any AEs, trial completion dates, the main reasons for discontinuation (if applicable).
The Investigator is obliged to provide direct access to the primary data and documents for the clinical trial specialists and/or authorized representatives (CRO) of the Sponsor, competent bodies' auditors, representatives of the insurance companies, Ethics Committees.

14 Finance and insurance

14.1 Funding

The trial is financed by the Sponsor through CRO. The relevant agreements will be concluded between CRO and every trial site before the trial initiation.

14.2 Insurance

The Principle Investigator of the research center is responsible for the safety of patients during this study. In case the patient develop adverse event, principle investigator and its personnel will provide medical care and do their best for the treatment.
When the patient agrees to participate in the trial, his participation in this study will be insured by [redacted] ([redacted]) in accordance with the Federal Law of April, 12 2010. No. 61-FZ "On circulation of medicines", Government Decree of the Russian Federation No.714 of September 13, 2010 "On approval of typical rules for compulsory insurance of the life and health of a patient involved in clinical trials of a medicinal product" and Government Decree of the Russian Federation No 393 of May, 18 2011 "On amendments to the typical rules for compulsory insurance of the life and health of a patient involved in clinical trials of a medicinal product".
In accordance with the Russian Federation Government Decrees No. 714 of 13.09.2010, No. 393 of 18.05.2011 "On approval of typical rules for compulsory insurance of the life and health of a patient involved in clinical trials of a medicinal product" the amount of the insurance payment under the contract makes:
a) in the event of death of the Insured Person - RUR 2 million. The benefit in mentioned extent shall be distributed throughout beneficiaries accordingly to theirs quantity share and share alike;
b) in the case of the Insured Person’s health impairment with the following sequelae:

- first class disability - 1.5 mln rubles;
- second class disability - RUR 1 million;
- third class disability - RUR 500 thousand;

c) in the case of the Insured Person's health impairment without any disability – not more than RUR 300 thousand.
Each patient will receive in person an original certificate of insurance and a reminder card with policy conditions and a plan of action in the case of injury. In order to preserve anonymity, personal data of each patient in the certificate of insurance will be replaced with an individual identification code of the patient which is assigned to her/him within the study according to the form established in the territory of the Russian Federation. In the case of harm to the health of a volunteer associated with the clinical study, the Insurance Company [redacted] shall reimburse all costs of required medical examination and care the need for which will arise as a result of direct exposure to the investigational medicinal product and reference product and/or medical procedures used according to the study protocol.
For additional information patients can refer to the insurance company office: [redacted]
The study does not specify any additional types of voluntary insurance or other options to grant treatment and / or compensation in the event of a patient's death or injury to the patient within the study.
The Sponsor shall not be liable for any loss, harm and/or injury caused to the patient, provided that such loss, harm and/or injury are induced by:

- Administration of the excluded medication within the study;
- Deviation on the part of the patient from the study protocol, study requirements and/or any instructions or guidelines that the study physician can give;
- Action or inaction of the third party in terms of proper response to adverse event or investigational drug reaction.

15 Publications

The information contained in this document is the property of the Sponsor, and its transfer to third parties is allowed only with the written permission of the Sponsor. This information may be accessed only by the investigators and the trial site personnel, members of the independent Ethics Committee, or health authorities responsible for the clinical trial control. Information about the trial, to the extent necessary to make a decision about provision of consent to participation, shall be provided to the volunteers, whom the Investigator plans to include into the trial.
The exclusive rights for this trial results belong to the trial Sponsor. No data from this trial may be presented or published without a prior written permission of the Sponsor.

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17 Appendices

17.1 Appendix 1. Claims

1. **DEFINITIONS**

Non-compliance with the requirements of investigational medicinal product (study product and reference product) or medical device use may include the following:

1) Claims related to the quality of the product/medical device such as

- any fault of quality and/or efficacy e.g. change of visual appearance, change of amount, damaged tablets/capsules, presence of particulate matter.
- any fault of the containers and outer packages e.g. surface defect, container leakage, broken syringe/plunger, missing contents, device malfunction.
- any fault of the labeling e.g. missing or illegible label.
- any falsification of the medicinal product or medical device e.g. suspected product mix-up, tampering or counterfeiting.

2) Problems related to the transportation of the product, such as damaged packaging for transport and/or damaged secondary packaging after receipt of the goods, a lack of quantity of medicinal product in the packaging for transport, inadequate transport conditions.

2. **PROCEDURE**

In case any of the non-compliances listed above are detected or information about these has been received, the prepared Non-compliance Report must be sent to the sponsor (principal investigator/clinical research manager) within 24 hours. Simultaneously local monitor or CRA of the respective study must be informed about the non-compliance. If possible, a photo of the affected material should be attached to the report. Affected material should be retained and stored according to the storage conditions label and/or returned to sponsor if requested by the Sponsor.

17.2 Appendix 2. Patient diary

Handed out at visits 1, 2, and 3

To be filled in by the physician:	*Patient diary hand-out visit:* ☐ *1* ☐ *2* ☐ *3*	*Patient number:* _____

1. Schedule of investigational drug administration between the visits

Day No.	Date	Have you taken Ferrum Lek®/ Maltofer® according to the medical prescription?	Commentaries and complaints
1 *	__/__/____	☐*Yes* ☐ *No*	
2	__/__/____	☐*Yes* ☐ *No*	
3	__/__/____	☐*Yes* ☐ *No*	
4	__/__/____	☐*Yes* ☐ *No*	
5	__/__/____	☐*Yes* ☐ *No*	
6	__/__/____	☐*Yes* ☐ *No*	
7	__/__/____	☐*Yes* ☐ *No*	
8	__/__/____	☐*Yes* ☐ *No*	
9	__/__/____	☐*Yes* ☐ *No*	
10	__/__/____	☐*Yes* ☐ *No*	
11	__/__/____	☐*Yes* ☐ *No*	
12	__/__/____	☐*Yes* ☐ *No*	
13	__/__/____	☐*Yes* ☐ *No*	
14	__/__/____	☐*Yes* ☐ *No*	
15	__/__/____	☐*Yes* ☐ *No*	
16	__/__/____	☐*Yes* ☐ *No*	
17	__/__/____	☐*Yes* ☐ *No*	
18	__/__/____	☐*Yes* ☐ *No*	
19	__/__/____	☐*Yes* ☐ *No*	
20	__/__/____	☐*Yes* ☐ *No*	
21	__/__/____	☐*Yes* ☐ *No*	
22	__/__/____	☐*Yes* ☐ *No*	
23	__/__/____	☐*Yes* ☐ *No*	
24	__/__/____	☐*Yes* ☐ *No*	
25	__/__/____	☐*Yes* ☐ *No*	
26	__/__/____	☐*Yes* ☐ *No*	
27	__/__/____	☐*Yes* ☐ *No*	
28	__/__/____	☐*Yes* ☐ *No*	

* Day 1 – Visit day.

2. Concomitant therapy

If you took any vitamins or medications at your discretion **together with Ferrum Lek® or MALTOFER ®,** please, put them down in the table below and report to your physician about them.

Product name	Date started	Date ended	Reason for administration

Please return the completed diary to the physician at the next visit