FONDAZIONE
ITALIANA
SINDROMI
MIELODISPLASTICHE

Clinical Protocol

Title: Early and low dose Deferasirox (3.5 mg/kg FCT) to suppress NTBI and LPI as early intervention to prevent tissue **iron** overload in lower risk **MDS**.

IRON – MDS

ID Study: FISM_IRON – MDS
EudraCT number: 2018-003542-17

INVESTIGATOR SPONSOR
Fondazione Italiana Sindromi Mielodisplastiche -ETS (FISiM-ETS)

COORDINATING INVESTIGATOR/S	Dr. Emanuele Angelucci, Genova, Italia
WRITING COMMITTEE AND SCIENTIFIC SUPPORT	Dr Mario Capasso, Napoli, Italia Prof Matteo Della Porta, Milano, Italia Dr. Gianluca Forni, Genova, Italia Prof Domenico Girelli, Verona, Italia Dr Ester Oliva, Reggio Calabria, Italia Dr Federica Pilo, Cagliari, Italia Prof. Valeria Santini, Firenze, Italia

Version and date of Protocol: Version 2.1, April 22, 2020

CONFIDENTIALITY STATEMENT

1. RESPONSIBILITIES

1.1. *Sponsor and data owner.*

✉ : Fondazione Italiana Sindromi Mielodisplastiche-ETS (FISiM-ETS), Via De' Poeti, 1/7 - 40124 Bologna, Italy
☎: +39 349 6754617
Email: segreteria@fismonlus.it

1.2. *Coordinating Investigator(s)*

✉ : Emanuele Angelucci, Ematologia, Ospedale Policlinico San Martino, Largo Rosanna Benzi 10, 16132 Genova, Italy
☎ :+39 010 5553651 Fax: +30 010 5556789
Email: emanuele.angelucci@hsanmartino.it, emnang@tin.it

1.3. *Writing Committee*

✉ : Mario Capasso, Dipartimento di Medicina molecolare e Biotecnologie mediche,Università degli studi di Napoli Federico II, Via Pansini 5, 80131 Napoli, Italy
☎ : +39 081-3737889
Email: mario.capasso@unina.it

✉ : Matteo Della Porta, Ematologia, Istituto Humanitas, Via Manzoni 56, 20089 - Rozzano, Milano, Italy
☎ : +39 02 82247668
Email: matteo.della_porta@hunimed.eu

✉ : Gianluca Forni, S.S.D. Microcitemia, anemie congenite e dismetabolismo del ferro, Ospedali Galliera, Mura delle cappuccine 14, 16128 Genova
☎ : +39 010 5634557
Email: gianluca.forni@galliera.it

✉ : Domenico Girelli, Dipartimento di Medicina, Università degli studi di Verona, Piazzale L. A. Scuro, 10, 37134 Verona, Italy
☎ :+39 0458124791 Fax: +39 0458027473
Email: domenico.girelli@univr.it

✉ : Federica Pilo, Ematologia. Ospedale Oncologico A. Businco, Azienda Ospedaliera Brotzu, via Edward Jenner sn, 09121 Cagliari
☎ : +39 339 6879269
Email: pilofederica@gmail.com

✉ : Ester Oliva, U.O.C. di Ematologia, Grande Ospedale Metropolitano"Bianchi-Melacrino-Morelli", Viale Europa,89133 Reggio Calabria (RC), Italy☎ : +39 0965.591791 Fax: +39 0965.349513
Email: estheroliva@hotmail.com

✉ : Valeria Santini, S.O.D. Ematologia, Azienda Ospedaliero Universitaria Careggi, Largo Brambilla 3, 50134 Firenze, Italy☎ : +39 055 7947476 Fax: +39 055 412098
Email: valeria.santini@unifi.it

1.4. *Biological Studies Coordinator*

✉ : Federica Pilo, Ematologia. Ospedale Oncologico A. Businco, Azienda Ospedaliera Brotzu, via Edward Jenner sn, 09121 Cagliari
☎ : +39 339 6879269
Email: pilofederica@gmail.com

1.5. Pharmacovigilance

✉ : Ufficio Farmacovigilanza, Uffici Studi FIL, Fondazione Italiana Linfomi Onlus, c/o Uffici PACTO, Spalto Marengo 44, 15121 Alessandria
☎: +39.0131.033156 Fax: +39 0131 263455
Email: drugvigilance@filinf.it

1.6. Start up

✉ : Sandra Mossuto FISiM ETS Via De' Poeti, 1/7 - 40124 Bologna, Italy
☎ : +39 3496754617
Email: segreteria@fismonlus.it

1.7. Study Coordinator

✉ : Sandra Mossuto FISiM ETS Via De' Poeti, 1/7 - 40124 Bologna, Italy
☎ : +39 3496754617
Email: segreteria@fismonlus.it

1.8. Coordinating Statistician

✉ : Mario Capasso, Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Via Pansini 5, 80131 Napoli, Italy
☎ : +39 081-3737889
Email: mario.capasso@unina.it

2. TABLE OF CONTENTS

3. INVESTIGATOR AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

_____

Investigator's Signature Date

_

Name of Investigator (Typed or Printed)

_____

Institution, Address*

_____

Phone Number*

Investigator-Sponsor Signature* Date
(where required)

_____

Name of Coordinating Investigator (Typed or Printed)

_____

Institution

* If the address or phone number of the investigator changes during the course of the study, written notification will be provided by the investigator to the sponsor and will not require protocol amendment(s).

4. SYNOPSIS

Study ID	**FISM_IRON – MDS**	
Eudract N°	**2018-003542-17**	
Title of the study	Early and low dose Deferasirox (3.5 mg/kg FCT) to suppress NTBI and LPI as early intervention to prevent tissue **iron** overload in lower risk **MDS**.	
Phase of the study	II	
Investigational product	DEFERASIROX FCT	
Protocol version	2	
Centers	About 12 Italian Hematology centers	
	Objective	Endpoint
Primary Objective and endpoints	Balance iron burden in one-year treatment in early phase of transfusion requirement by low dose (3.5 mg/kg) DFX-FCT (prevention of iron overload) as demonstrated by hepatic iron concentration.	Change of hepatic iron from the baseline according to baseline hepatic iron level: For patients with baseline LIC ≤ 5 mg/g dry weight (dw) ± 1.5 mg/g dw. For patients with baseline LIC >5 mg/g dw ±20% as demonstrated by R2- MRI (test performed in a 1.5 tesla MRI machine and analyzed following R2 method – Baseline versus EOS. The corresponding secondary efficacy variable will be the absolute change in hepatic iron concentration EOS versus baseline.
Secondary Objectives	Objectives	Secondary Endpoints
Descriptive-observational objective	Definition of iron overload (including serological markers and MRI definition of iron loading in liver, tissue reactive iron species and oxidative stress in MDS at beginning of transfusional history.	Absolute values of serum ferritin, transferrin saturation, NTBI, LPI, liver MRI and oxidative stress at baseline.
	Efficacy	Absolute change in hepatic iron concentration EOS versus baseline.
	On year evolution of iron overload serologic markers	Absolute and relative changes in serum ferritin and transferrin saturation from baseline to every visit during the whole treatment period.

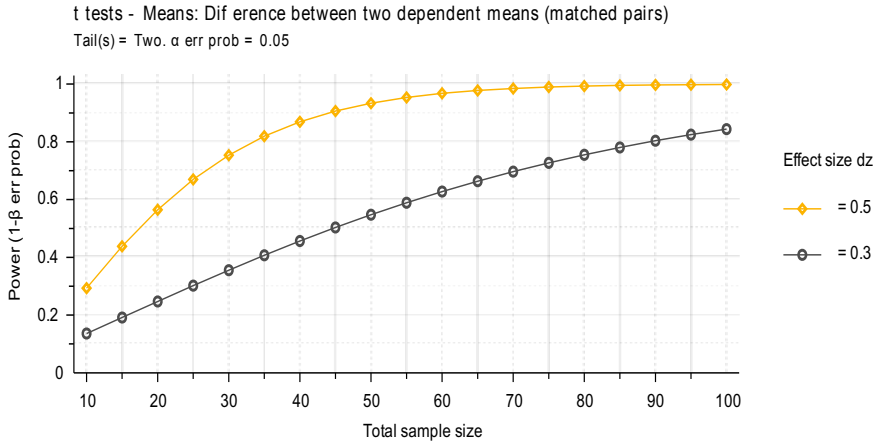
	Presence and quantitative evolution of toxic serum iron forms (iron tissue reactive species) under low dose DFX therapy.	Proportion of patients with NTBI > normal values and/or LPI > normal values at end of study vs baseline. Changes in NTBI and LPI from baseline to every visit during the whole treatment period.
	Verify if regular suppression of the "free iron forms" prevent accumulation of tissue iron.	Relationship between NTBI and LPI with serum ferritin and liver and pancreas iron overload (MRI) at end of study versus baseline
	Evaluate the overall safety of deferasirox FCT formulation in patients with lower risk MDS at the beginning of their transfusional history	Overall safety, as measured by frequency and severity of reported AEs and SAEs and changes in laboratory values from baseline: serum creatinine, creatinine clearance ALT, AST, complete blood count (platelets, RBC and WBC) and total direct and indirect bilirubin).
	Leukemic transformation (including progression to leukemia or higher rIPSS scores)	Proportion of patients with a disease progression (progression defined as a transition into a higher MDS risk group based on revised IPSS scoring or progression to AML) Time to progression (defined as above) or to leukemia transformation.
	Hemopoietic response	Percentage of patients with hematologic improvements in term of erythroid response following IWG 2006 criteria. Time to reach transfusion dependence defined as > 2 PRBC units/months for 3 months for patients with pre-transfusional Hb < 9.0 g/dl (or <10 g/dl for patients with cardiac disease) or a total number of PRBC units received = 20 from baseline. Absolute reticulocytes count from baseline to every visit during the whole treatment period. Absolute values and evolution of serum transferrin receptor, GDF11, GDF15 and erythroferrone from baseline to every visit during the whole treatment period. Evaluation of transfusional ratio: number of units received in a

		predetermined period/ mean pre-transfusional Hb level. Compared with other studies on general population (study and FISiM registry). Patients receiving concurrent rHuEpo and other disease modifying agents will not be considered for hemopoietic response.
	Costs analysis	Cost and outcome with low dose in early chelation vs. chelation treatment in MDS patients with transfusional iron overload in accordance with either local or international guidelines
Exploratory	Study of biological cellular damage by iron toxicity before and during treatment	MDA values during the study and relationship to NTBI and LPI values
Study design	• This is an open-label, single arm, phase II, study designed to look whether early intervention with low dose DFX improves clinical outcome of patients with MDS. • *Efficacy of early - low dose DFX FCT to prevent iron accumulation regularly suppressing reactive oxygen species.* • *Safety of early low dose DFX FCT.* • *Exploratory: possibility to guide chelation therapy by NTBI/LPI.* • *One arm* • *Fixed dose* • *Blinding: Open* • *Structure: Single Group* • *Randomized: Non-randomized* • *A standard pre-transfusion Hemoglobin level will be indicated (*Hb threshold for transfusion Hb 9 g/dl or 10 g/dl for patients with cardiac disease) *to be maintained during the study year. Pre transfusion hemoglobin level and transfusions date and frequencies will be recorded. A transfusional ratio will be determined: number of units received in a predetermined period/ mean pre-transfusion Hb level*	
Duration of the study	Enrolment 1 year. Per protocol treatment: 1 year. Total duration 2 years	
Number of patients	60 (50 + 10 for 20% drop out rate)	
Population	Adult MDS patients (≥18 years). Very low, low and intermediate risk group by revised-IPSS. Regularly transfused patients with low PRBC transfusion burden (less than 2 units/months for 3 consecutive months) at beginning of their transfusion story (total number of PRBC units received 5-20). Patients should not meet the published criteria for transfusion dependency (2 PRBC units /month for three consecutive months).	
Inclusion criteria	Study population: • Diagnosis: Adult Myelodysplastic Syndrome (≥18 years). • Revised IPSS: very low. low – intermediate.	

	• Having received 5-20 packed red blood cell units • Serum ferritin ≥300 ng/ml • Transferrin saturation ≥60% • Chelation naïve • Capability to provide informed consent
Exclusion criteria	• Patients aged <18 years old • Higher risk (revised IPSS) MDS (Intermediate 2, high) • Cumulative transfusion story of > 20 packed red cell units • Creatinine Clearance (CrCL): <60 ml/min. Patients with CrCl of 40-60ml/min will be included only individually if no other renal risk factors are present. • Serum creatinine >2 x ULN at screening. If borderline serum creatinine will be measured within 7-10 days and the mean value will be used for eligibility criteria. • Significant proteinuria as indicated by a urinary protein/creatinine ratio > 0.5 mg/mg in a non-first void urine sample (or alternatively in two of three samples obtained for screening). • ECOG performance status >2. • Left ventricular ejection fraction < 50% by echocardiography • A history of repeated hospitalization for congestive heart failure. • Systemic diseases that would prevent study treatment (e.g. uncontrolled hypertension, cardiovascular, renal, hepatic, metabolic, etc.) • Clinical or laboratory evidence of chronic Hepatitis B or Hepatitis C (definition of chronic hepatitis follows EASL 2017 criteria). • History of HIV positive test result (ELISA or Western blot). • Treatment with systemic investigational drug within 4 weeks or topical investigational drug within 7 days of study start. • ALT or AST over 3 times superior to ULN at screening. • ANC < 500/ microL • Platelets transfusion dependency • Total bilirubin over 1.5 times superior to ULN at screening (patients with Gilbert syndrome are allowed to enter the study) • Diagnosis of Child score C liver cirrhosis. • Patients participating in another clinical trial other than an observational registry study. • Patients with a history of another malignancy within the past 3 years, with the exception of basal skin carcinoma or cervical carcinoma in situ or completely resected colonic polyps carcinoma in situ. • History of non-compliance to medical regimens, or patients who are considered potentially unreliable and/or not cooperative. • Presence of a surgical or medical condition which might significantly alter the absorption, distribution, metabolism or excretion of study drug. • Pregnant, intending-to-become pregnant, or breast-feeding patients. • Women of potential maternity age who do not agree to practice effective contraceptive methods for the entire study duration. • History of drug or alcohol abuse within the 12 months prior to enrollment.

<table>
<tr><td></td><td>

Hypersensitivity to the active substance or to any of the excipients.
Inability to provide a valid informed consent.

</td></tr>
<tr><td>Study treatment and response criteria</td><td>DFX FCT 3.5 mg/kg.
Response criteria: prevention of iron overload as measured by liver iron concentration (MRI).</td></tr>
<tr><td>Assessments schedule</td><td>MONTHLY</td></tr>
<tr><td>Centralized analyses</td><td>
<table>
<tr><th>TEST</th><th>REQUIRED</th><th>QUANTITY</th></tr>
<tr><td>NTBI, LPI, Epcidine, MDA</td><td>YES see schedule</td><td>7</td></tr>
<tr><td>Serum transferrin receptor, GDF11 e 15, Erythroferrone.</td><td>YES see schedule</td><td>5</td></tr>
<tr><td>NGS (molecular tumor analysis)</td><td>YES see schedule</td><td>2</td></tr>
<tr><td>MRI liver scan</td><td>YES Baseline and EOS</td><td>2</td></tr>
</table>
</td></tr>
<tr><td>Statistical considerations</td><td>


Including 50 subjects in the study, we can reach almost 60% power to detect small effect size 0.30 (with p-value of 0.05) whereas, we have almost 100% to detect moderate effect size of 0.50 (with p-value of 0.05).
On the basis of previous experience, the low dosage, the early intervention and the FCT preparation a 20% drop out rate is expected (10 additional patients to be enrolled)

Methods: The power test was performed by G power 3.1 software by using the t test Means: Difference between two dependent means (matched pairs).

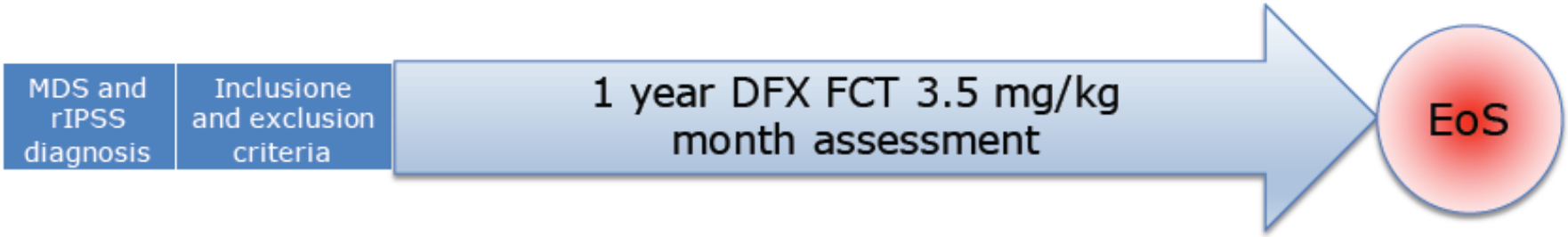
As demonstrated by pour sample size analyses, we have enough power to detect an effect size between 0.3 and 0.5 considering a potential error of 0.05 which is largely accepted from scientific community.
</td></tr>
</table>

5. LIST OF ABBREVIATIONS AND GLOSSARY OF TERMS

ABBREVIATION	TERM
AE	Adverse Event
ALT (SGPT)	ALanine Transaminase (Serum Glutamic Pyruvic Transaminase)
AP	Activity population
AST (SGOT)	ASpartate Transaminase (Serum Glutamic Oxaloacetic Transaminase)
β-HCG	beta-Human Chorionic Gonadotropin
BSA	Body Surface Area
CBC	Complete Blood Cell
CNS	Central Nervous System
CR	Complete Response
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DNA	Deoxyribonucleic acid
DSMC	Data Safety Monitoring Committee
EASL	European Association for Study of the Liver
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EOR	End of treatment Response
EP	Efficacy population
ERC	Ethics Review Committee
FCBP	Female of Child Bearing Potential
FISiM-ETS	Fondazione Italiana Sindromi Mielodisplastiche-ETS
FPFV	First Patient First Visit
GCP	Good Clinical Practice
GDF15	Growth Differentiation Factor15
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IMP	Investigational Medicine Product
IP	Investigational Product
ITT	Intention To Treat
IV	Intra Venous
LCI	Labile Cellular Iron
LDH	Lactic DeHydrogenase
LIC	Liver iron concentration
LPFV	Last patient Fist Visit
LPI	Labile Plasma Iron
LVEF	Left Ventricular Ejection Fraction
MDA	Malonildialdehyde
MRI	Magnetic Resonance Imaging
NGS	New Gereration Sequencing
NTBI	Non Transferrin Bound Iron
ORR	Overall Response Rate
OS	Overall Survival
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PFS	Progression Free Survival
PK	PharmacoKinetics
PR	Partial Response
PS	Performance Status
SAE	Serious Adverse Event

SD	Stable Disease
SP	Safety population
SUSAR	Suspected Unexpected Serious Adverse Reaction
Tsat	Transferrin Saturation
ULN	Upper Limit of Normal
WHO	World Health Organization

6. STUDY FLOW CHART



7. INTRODUCTION. AIM OF THE STUDY

Iron chelation therapy in MDS patients receiving transfusion therapy has been developed on the basis of the large experience in transfusion dependent Thalassemia. However Thalassemia is a different clinical scenario compared to MDS for several different reasons. This study protocol has been developed to focus the specific setting of elderly MDS patients, inside the registered indications. This protocol has been designed to look whether early intervention with low dose DFX improves clinical outcome of patients with MDS.

8. BACKGROUND

8.1. Disease background

Myelodysplastic syndromes (MDS) are a wide range of syndromes involving the hemopoietic stem cells and clinically expressing with peripheral cytopenias in different forms. Cytopenias mainly involve the erythropoietic line and has the clinical picture of anaemia. The clinical course of untreated MDS is characterized by worsening of the clinical picture and by a variable risk of developing secondary acute leukaemia (range from few months to several years). MDS patients are classified following the International Prognostic Scoring System (IPSS)(1) developed in 1997 by Peter Greenberg and revised in 2012 (rIPSS)(2) predicting leukaemia free survival and overall survival.

MDS and iron overload

Most of the MDS patients require regular packed red blood cells (PRBC) transfusions and finally most of them became transfusion dependent. One of the unavoidable consequences of anaemia and of transfusion requirement is iron overload which has been proved to be deleterious for different categories of patients including MDS patients (3–7).
Little is known on anaemia contribution to iron overload/toxicity in the specific setting of MDS but a worsening impact is likely(8).

Iron overload and iron toxicity in MDS

Iron related tissue and organ damage (and finally mortality) has been, so far, mainly related to what has been developed in the field of children, adolescent and young adults with thalassemia major(9). Nevertheless some papers(5,10–16) and a meta-analyses(17) have related MDS patients survival to serum ferritin level which is a very well known but a-specific marker of iron overload

Tissue and organ damage has been directly and strictly connected to the amount of tissue iron deposition (i.e. a “bulky” disease). In other words, tissue/organ damage was directly related and dependent to the amount of metal accumulation. All the studies performed in the last years (again mainly in transfusion dependent thalassemia) linked survival to markers of iron accumulation (indirect markers as serum ferritin and cardiac and liver MRI or direct markers as liver iron concentration in biopsy specimens).

New concept of iron toxicity

There are two major limitations to this interpretation:

1. The clinical scenario of elderly MDS patients is different from the “pivotal” situation of thalassemia major because of age (including tissue reparative mechanisms), comorbidities and underlying disease(18,19).
2. Recent data support the concept that iron disease is not only a “bulky” disease exclusively secondary to iron accumulation but rather a toxic disease in which tissue damage is due to toxic iron forms (tissue reactive iron i.e. NTBI, LPI etc.) present in plasma since early phase of transfusion therapy or even before (20,21).

Particularly this second point require detailed explanation:
Tissue iron toxicity is today expressed by the following formula developed by Thomas Coates (22)

$$\text{Fe Toxicity} = \Sigma\text{tissue reactive iron} \times \text{genetics} \times \text{environmental factors} \times \Delta\ \text{time}$$

Where:

Σ tissue reactive iron	= tissue toxicity sums (Σ), ROS generation
Genetics	= The marrow pathology, differences in iron transport, antioxidant defense mechanisms
Environmental factors	= nutritional status, blood transfusions, drugs that may modulate iron toxicity, comorbidities (i.e. viral infections, ecc), Administration of chelating agents
Δ time	= time of exposition

It is clear from this formula that:

a) Duration of exposure to tissue reactive iron is the key point to predict the severity of target tissues damage
b) Classic biomarkers of iron overload (serum ferritin, MRI etc.) can be interpreted as biomarkers of duration of exposure.
c) Tissue damage starts much sooner than iron accumulation becomes evident, as demonstrated by standard parameters like MRI and serum ferritin(21,23,24).
d) Environmental factors and individual genetics are a substantial difference between individuals and diseases (between young patients with hemoglobinopathies and elderly lower risk MDS patients).

It has been widely demonstrated that toxic free iron species (NTBI and LPI) appear in the serum only once iron binding capacity is saturated in a rate over 60-70%(25–27). Notably these iron fractions are chelatable and can be removed from circulation by a chelator (28).

Classical approach to chelation therapy in MDS (29,30) is today overtaken by above reported new model. Even the conventional safety issue of minimal serum ferritin level is outdated even in the delicate category of children with thalassemia major (31,32). In the phase II KALLISTO trial no significant or unexpected Deferasirox side effects were reported in a population of un-transfused lower risk MDS patients (33).

8.2. Mechanism of iron accumulation and cellular iron damage

Cellular iron influx and accumulation is caused, in a controlled way, by uptake of transferrin-iron by transferrin receptors in cells expressing on the membrane transferrin receptors (mainly liver) (22,27,34,35). In addition, non-transferrin-bound-iron (NTBI) (36) and Labile Plasma Iron (LPI), a directly chelatable form of NTBI, are readily taken up by cells, by an uncontrolled mechanism through calcium channels, leading to expansion of the labile cellular iron pool (LIP) (28,37,38). This mechanism is the only one in tissue/cells lacking or with minimal transferrin receptor expression (heart, pancreas).

The liver is the main site of iron storage and accumulation(27). In transfusion dependent thalassemia it has been demonstrated that hepatic iron concentration is proportional to whole body iron stores up to a hepatic iron concentration of 25 mg/g dry weight in absence of cirrhosis. The total body iron store (mg/kg) can be calculated multiplying hepatic iron concentration (mg/g dry weight) x 10.6 with a standard error of ±7.3(39).

In physiologic situation there is a delicate intracellular balance between iron functional and storage pool maintaining Labile Iron Pool levels within a 0.5–1.5 μM physiological range by an iron-sensing-transducing machinery that coordinately regulates uptake vs storage so as to support Fe utilization and minimize Fe-O-driven oxidations .

Uncontrolled cellular iron uptake leads to increment of cellular labile iron pool. An excessive rise in LPI can promote the generation of reactive Oxygen species (ROS) by reacting with respiratory Oxygen intermediates and thereby override the cellular antioxidant defences and chemically damage cell components and associated functions (40). This mechanism has as final effect, throughout a complex biochemical cascade of cellular damage and apoptosis(41,42).

Prevention of accumulation means therefore regular suppression of reactive oxygen species and consequent tissue damage prevention.

Subsequently a rational approach to iron chelation therapy is to use a chelator at the minimum efficient dose able to completely and persistently suppress tissue reactive iron species to avoid tissue damage and prevent iron accumulation (43).

This approach is theoretically very important in MDS patients because of the major sensitivity of several tissues (including vascular) due to aging, comorbidities and absence or deficiency of tissue reparative mechanism (44,45). A study on the specific pathogenic role of LPI in MDS is ongoing (20).

In the setting of hemopoietic stem cells, experimental data demonstrates a detrimental effect of reactive oxygen species on hemopoietic stem cell maturation and self-renewal capability (46–49). Clinical data are in accordance with these experimental data (50–54). Moreover a debate is on-going on the possible role of iron related reactive oxygen species in accelerating (favoring) MDS progression to acute leukemia (55).

Consistent reports of improvement of hemopoieisis in transfusion dependent MDS patients receiving iron chelation (51,55–57) are in accordance with removal by chelation of toxic factors compromising hemopoieisis, more that the disease itself.

Laboratory definition of tissue reactive iron has been a challenge for several years (36) and is today possible with a standardization on-going (25); however it has been clearly demonstrated that toxic free iron form emerge in plasma only once serum transferrin is saturated over 60-70% (22,25).

8.3. Current therapies for iron overload in lower risk MDS

Iron chelation therapy by deferasirox has been approved for low and intermediate-1 IPSS patients worldwide and its clinical benefit has been uniformly proved by several retrospective studies, prospective case control, registry and prospective (58) not randomized clinical studies. A recent meta-analysis confirmed the clinical value of iron chelation for lower risk MDS patients(59). A large, confirmatory Canadian prospective registry study (60) has been published after the meta-analysis publication The prospective, randomized placebo control study is expected to provide definite information by the end of 2018 (ClinicalTrials.gov Identifier: NCT00940602).

Almost all the scientific societies published guidelines and regulatory agencies have indicated /approved chelation therapy in MDS after that a condition of chronic post transfusional iron overload has been achieved. Treatment is therefore determined by iron accumulation that is considered a mandatory prerequisite for iron toxicity (and not by iron toxicity per se). With minor variations, threshold for starting iron chelation has been identified adopting that in place for transfusion dependent thalassemia even though levels of accumulation historically associated with organ damage (based on data generated in the Thalassemias) are infrequent in MDS: 20 units of packed red blood cells and/or in a serum ferritin level of 1000 ng/ml (29,30) even if in real world clinical practice a higher threshold is common.

On the same basis indication to lower risk IPSS categories has been established on the idea that higher risk MDS patients live not enough to let dangerous iron accumulation (61).

Deferasirox has been proved to suppress toxic – redox active iron species even if the minimum necessary dose has not been explored (56,62,63).

Another iron chelator is actually approved worldwide for iron chelation in MDS: Deferoxamine, Desferal®. Deferoxamine modality of administration and half-life are not appropriate to patients with MDS. Another thalassemia licensed oral iron chelator (Deferiprone, Ferriprox®) is not approved in EU and US for MDS patients and limited MDS-literature is available (64).

8.4. Study drug (Mechanism of Action, Absorption, Distribution, Metabolism and Excretion)

Deferasirox (ICL670, Exjade®) is an N-substituted bis-hydroxyphenyl-triazole, a representative of a new class of tridentate iron chelators (65) that has been developed by Novartis for treating transfusional iron overload. Two molecules of Deferasirox form a complete complex with Fe3+. The high potency of deferasirox in mobilizing tissue iron and promoting iron excretion was demonstrated both in vitro and in vivo model systems (65). Deferasirox is eliminated from the body by hepatic glucuronidation and biliary excretion.
Preclinical studies also revealed that deferasirox did not affect fertility and it is neither teratogen nor carcinogenic.
To date, deferasirox has been approved in more than 100 countries, including the European Union, the USA, Switzerland, and Japan for the treatment of chronic iron overload due to blood transfusions in adult and pediatric patients. Within this indication, deferasirox is approved for use in transfusion-dependent MDS patients, based on data demonstrating efficacy in reducing hepatic iron concentration and serum ferritin.
Detailed information on preclinical and clinical evaluation of deferasirox is provided in the SmPC.
Deferasirox was first released as a dispersible tablet (DT) for oral suspension which facilitates administration of the appropriate quantity of drug substance to pediatric and adult patients.
Bioavailability studies with deferasirox dispersible tablets indicated that absorption is increased with food, and this is dependent on the fat content and the timing of food intake. Deferasirox dispersible tablet was taken on an empty stomach at least 30 minutes prior to food in clinical studies; this is also the recommendation in the label.

Deferasirox has demonstrated acceptable safety and tolerability in adult and pediatric patients with transfusional iron overload (51,66,67). The most frequent reactions reported during the first year of treatment with deferasirox in adult and pediatric patients included gastrointestinal (GI) disturbances in about 26% of patients (mainly nausea, vomiting, diarrhea, or abdominal pain), and skin rash in about 7% of patients. These reactions were dose-dependent, mostly mild to moderate, generally transient and mostly resolved even if treatment was continued. In addition, there have been rare reports of upper GI hemorrhage and/or ulceration in patients receiving deferasirox.
There have been very occasional post-marketing reports of erythema multiforme, leukocytoclastic vasculitis and hypersensitivity reactions (including anaphylaxis and angioedema). Alopecia, usually comprising thinning of the hair, has been occasionally reported in patients receiving deferasirox.
Mild, non-progressive increases in serum creatinine, mostly within the normal range, occurred in about 36% of patients during the first year of treatment. These were dose-dependent, often resolved spontaneously and were sometimes alleviated by reducing the dose. Rare cases of acute renal failure, defined as a serum creatinine increases ≥ 2 ULN, and usually reversible after treatment interruption (and rarely a brief course of hemodialysis), have been reported following the prescription use of deferasirox.
Elevations of liver transaminases were reported as an adverse reaction in about 2% of patients. These were not dose-dependent and most of these patients had elevated levels prior to receiving deferasirox. Elevations of transaminases **>**10 ULN were uncommon (0.3%). There have been post-marketing reports of hepatic failure, mostly in patients with severe baseline liver disease.
There have been reports of cytopenias, mostly in patients with pre-existing blood disorders which are frequently associated with failure of the bone marrow to produce sufficient amounts of blood cells.
High frequency hearing loss and lenticular opacities (early cataracts) have been uncommonly observed in patients treated with deferasirox. As with other iron chelator treatment, the risk of toxicity of deferasirox may be increased when inappropriately very high doses are given in patients with a low iron burden or with serum ferritin levels that are only slightly elevated. Recent data do not confirm these ocular and hearing toxicity in adults (68).

In summary, deferasirox is a once-daily oral iron chelator that has been developed for treating transfusional iron overload, with demonstrated efficacy in the reduction or maintenance of body iron stores, and an acceptable safety profile.

Recently Deferasirox film coated tables (DFX FCT) has been released (69) and is progressively substituting the water dispersible tables.

Deferasirox FCT can be taken in a single step: swallowed whole with water or other beverages, either on an empty stomach or with a light meal (<7% fat and ~250 calories) (69). Deferasirox DT, in contrast, requires administration on an empty stomach at least 30 minutes prior to a meal (69). When taken with a low-fat meal, there is a modest effect on the bioavailability and maximum plasma concentration of deferasirox FCT (reductions of 11% and 16%, respectively) (69).

Unlike deferasirox DT, deferasirox FCT does not contain lactose or sodium lauryl sulfate; this aspect may result in fewer GI side effects with deferasirox FCT compared with deferasirox DT (70). Like deferasirox DT, it is preferable to administer deferasirox FCT at the same time each day.

It is important to note that the dose for deferasirox FCT is approximately 30% lower, rounded to the nearest whole tablet, because the bioavailability of deferasirox FCT is higher than that of deferasirox DT. Deferasirox FCT is available in 90, 180, and 360 mg tablets. Deferasirox FCT may be crushed and mixed with soft foods, such as yogurt or apple sauce (69).

A recently published Phase II study (71) compared deferasirox DT with deferasirox FCT in patients with TDT or MDS (very low, low, or intermediate risk) who had iron overload and required deferasirox

DT (≥30 mg/kg/day for those with TDT or ≥20 mg/kg/day for those with MDS). Safety, GI side effects, palatability, satisfaction, and compliance were also assessed. The results of this study showed that the FCT formulation of deferasirox was well tolerated. In patients with prior deferasirox DT exposure, fewer GI AEs were seen with deferasirox FCT than with deferasirox DT. Compliance of patients receiving deferasirox FCT was enhanced, and these patients continued on treatment for longer times. More patients were satisfied with the new deferasirox FCT formulation than with the deferasirox DT formulation at all visits. Patient-reported outcomes were also improved with deferasirox FCT compared with deferasirox DT (71).

Deferasirox film coated tables (DFX-FCT) is the study drug of this study.

8.5. Complete and on-going clinical experiences with study drug

Deferasirox is registered for the treatment of adult and pediatric patients with chronic transfusional iron overload.

Thousands of pediatric and adult patients aged 2 to 80 years were enrolled in randomized controlled trials evaluating the safety and efficacy of deferasirox in the treatment of transfusional iron overload. Thousands of patients have received deferasirox in prospective studies and on clinical indication since its release. More than 1500 MDS patients have been included in clinical studies, which included iron chelation with DFX. The studies showed deferasirox to effectively chelate iron in patients with transfusional iron overload as demonstrated by decreases of hepatic iron concentration and serum ferritin (51,66,67,72,73).

The efficiency of deferasirox DT in chelating iron appears to be constant at all doses ranging from 5 to 40 mg/kg/day (3.5 to 28 mg/kg/day for the FCT formulation) and is not affected by age, gender, baseline LIC or underlying anemia (69).

Deferasirox film coated table (DFX-FCT) has been recently registered for showing an improved safety profile particularly regarding gastro-intestinal side effect (71).

9. STUDY RATIONALE

The scientific rationale for this study is the evolving understanding that iron-induced tissue damage is not only a process of progressive bulking of organs through high-volumes iron deposition, but also a reactive iron species related “toxic” damage.
Iron mediated damage can occur prior reaching high iron storage thresholds derived from thalassemia major setting, free toxic iron species being already present when transferrin saturation >60-70% (25); therefore a timely early adoption of iron chelation may be of benefit before overt iron overload is seen.

Our hypothesis is that early and low dose DFX-FCT is better tolerated and is able to prevent iron accumulation and consequently tissue iron related damage, by consistently suppressing iron reactive oxygen species (NTBI and LPI).

If this hypothesis is confirmed this approach could contribute to an improvement of clinical practice of patients managements. Additionally this approach might also be a contribute in preventing future iron overloaded related complication, in this already frail and co-treated patient population.

10. STUDY OBJECTIVES

10.1. Primary objective

Balance iron burden in one-year treatment in early phase of transfusion requirement by low dose (3.5 mg/kg) DFX-FCT (prevention of iron overload) as demonstrated by MRI (R2) documented hepatic iron concentration.

10.2. Secondary objectives

- Descriptive –observational objective: Definition of iron overload (including serological markers and MRI definition of iron loading in different tissues: liver, pancreas), tissue reactive iron species and oxidative stress in MDS at beginning of transfusional history (descriptive, observational).
- On year evolution of iron overload serologic markers
- Presence and quantitative evolution of toxic serum iron forms (iron tissue reactive species) under low dose DFX therapy.
- Verify if regular suppression of the “free iron forms” prevent accumulation of tissue iron.
- Evaluate the overall safety of deferasirox FCT formulation in patients with lower risk MDS at the beginning of their transfusional history
- Leukemic transformation (progression to leukemia or higher rIPSS scores)
- Hemopoietic response
- Costs analysis

10.3. Exploratory

Study of biological cellular damage by iron toxicity before and during treatment

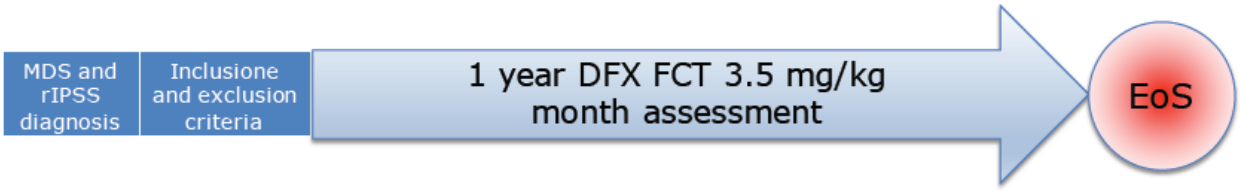
11. STUDY DESIGN

11.1. Overview of study design

This is an open label, single arm, phase II, study.
The study includes a screening period (in which patients eligibility will be assessed) followed by a 1-year treatment period during which monthly visits will take place to evaluate safety and efficacy parameters (total 16 visits + 1 screening visit).
Study treatment with low dose (3.5 mg/kg/day) DFX-FCT will be given to all enrolled patients at the beginning of their transfusional history

Dose activity will be monitored by regular NTBI /LPI suppression and by prevention of iron accumulation as demonstrated by hepatic iron concentration, comparing baseline versus end of study iron concentration. It has been clearly demonstrated that liver iron concentration is proportional to total body iron stores (LIC x 10.6 = total body iron store in mg/kg)(39)

A standard pre-transfusional Hemoglobin level will be indicated (Hb threshold for transfusion Hb 9 g/dl or 10 g/dl for patients with cardiac disease) to be maintained during the study year. Pre transfusional hemoglobin level and transfusions date and frequencies will be recorded. A transfusional ratio will be determined: number of units received in a predetermined period/ mean pre-transfusional Hb level



11.2. Number of patients

A total of 60 patients will be enrolled in the study: 50 +10 for a planned 20% drop out rate (drop our rate estimated on others DFX DT – MDS prospective studies).

11.3. Duration of the study

Patients will be recruited over 12 months and followed for 1 year treatment phase.
The anticipated study dates (start / end) are:

- Total accrual period (months/years):12 months
- The duration of the treatment period is 12 months
- End of the study: last patient last visit.
- The Final Study Report will be provided after the end of the Study.

12. STUDY POPULATION

12.1. Inclusion criteria

- Diagnosis: adult Myelodysplastic Syndrome (=> 18 years).
- Revised IPSS: very low. low – intermediate.
- Having received 5-20 packed red blood cell units
- Serum ferritin >300 ng/ml
- Transferrin saturation >60%
- Chelation naïve
- Ability to provide informed consent.

12.2. Exclusion criteria

- Patients aged <18 years old
- Higher risk MDS (Intermediate 2, high)
- Cumulative transfusional history of > 20 packed red cell units
- To be definitely classified as transfusion depended following published criteria (74) (having received 2 packed red cell units /months for three consecutive months)
- Creatinine Clearance (CrCL): <60 ml/min. Patients with CrCl of 40-60ml/min will be included only individually if no other renal risk factors are present.
- Serum creatinine >2 x ULN at screening.
- Serum creatinine will be measured at screening (repeatable)
- Significant proteinuria as indicated by a urinary protein/creatinine ratio > 0.5 mg/mg in a non-first void urine sample at screening (repeatable).

- ECOG performance status >2.
- Left ventricular ejection fraction < 50% by echocardiography
- A history of hospitalization for congestive heart failure.
- Systemic diseases which would prevent study treatment (e.g. uncontrolled hypertension, cardiovascular, renal, hepatic, metabolic, etc.)
- Clinical or laboratory evidence of active Hepatitis B or Hepatitis C (see EASL criteria).
- History of HIV positive test result (ELISA or Western blot).
- Treatment with systemic investigational drug within 4 weeks or topical investigational drug within 7 days of study start.
- ANC < 500/ microL
- Platelets transfusion dependency
- ALT or AST >3 x ULN at screening.
- Total bilirubin >1.5x ULN at screening (patients with Gilbert syndrome are allowed to enter the study).
- Diagnosis of liver cirrhosis child score C.
- Patients participating in another clinical trial other than an observational registry study.
- Patients with a history of another malignancy within the past five years, with the exception of basal skin carcinoma or cervical carcinoma in situ or completely resected colonic polyps carcinoma in situ.
- History of non-compliance to medical regimens, or patients who are considered potentially unreliable and/or not cooperative.
- Presence of a surgical or medical condition which might significantly alter the absorption, distribution, metabolism or excretion of study drug.
- Pregnant, intending-to-become pregnant, or breast-feeding patients.
- Women of potential maternity age who do not agree to practice effective contraceptive methods for the entire study duration.
- History of drug or alcohol abuse within the 12 months prior to enrollment.
- Hypersensitivity to the active substance or to any of the excipients.
- Inability to provide a valid informed consent.

13. PATIENTS ENROLLMENT

13.1. Informed consent

The Investigator(s) must obtain informed consent of a patient or his/her designee prior to any study related procedures as per Good Clinical Practices (GCP). Documentation that informed consent occurred prior to the patient's entry into the study and of the informed consent process should be recorded in the patient's source documents. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his/her disease. Subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. The subject or legally acceptable representative will be given sufficient time to read the informed consent form and the opportunity to ask questions. After this explanation and before the entry to the study, consent should be appropriately recorded by means of either the subject's or his/her legally acceptable representative's dated signature. After having obtained the consent, a copy of the informed consent form must be given to the subject.
If the subject or legally acceptable representative is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and personally date and sign the informed consent form after the oral consent of the subject or legally acceptable representative is obtained.
The original consent form signed and dated by the patient and by the person consenting the patient prior to the patient's entry into the study must be maintained in the Investigator's study folder and a

copy given to the patient. In addition, if a protocol is amended and it impacts on the content of the informed consent, the informed consent must be revised. Patients participating in the study when the amended protocol is implemented must be re-consented with the revised version of the informed consent. The revised consent form signed and dated by the patient and by the person consenting the patient must be maintained in the Investigator's study folder and a copy given to the patient.

13.2. Patient registration and data collection

Following confirmation of eligibility and written informed consent, patients should be registered online in the dedicated section of CRF website.
Each patient will be assigned a sequential patient number by the investigator. Once assigned to a patient, a patient number will not be reused. Subject identification numbers of patients who did not meet the eligibility criteria or did not start treatment will not be replaced.
For a given patient, patient number will not be changed throughout the entire study

14. TREATMENT

14.1. Drug 1: Deferasirox FCT

The investigational study drug used in this trial is deferasirox as film coated tablets for oral use (DFX-FCT)

14.1.1. Supplier(s)

Not applicable. The study drug will be prescribed to patients as part of routine clinical practice within its approved indication.

14.1.2. Preparation and administration

Self-administration following Physician prescription.
DFX FCT should be swallowed whole with some water on an empty stomach or after a light meal. For patients who are unable to swallow whole tablets, the FCT may be crushed and administered by sprinkling the full dose onto soft food, e.g. yogurt or apple sauce. The dose should be immediately and completely consumed, and not stored for future use.

Patient will be prescribed with an appropriate number of DFX FCT from a choice of 90 mg, 180 mg, and 360 mg strengths, based on the patient's weight calculated overall daily dose.

14.1.3. Receipt of study drug

NA

14.1.4. Storage and handling

NA

14.1.5. Unused study drug supplies

Patients will be instructed to take the assigned amount of drug and asked to report the exact drug dose assumed and any deviation.

14.2. Treatment schedule and design

14.2.1. Single arm (DFX-FCT 3.5 mg/kg/day)

Having completed the screening period, patients will be assigned to a fixed dose of 3.5 mg/kg/day of DFX FCT.

14.3. Premedication

No need of premedication

14.4. Prophylactic measures

14.4.1. Prevention of infections

As per clinical practice at investigator discretion. List of drug interaction is provided in the Summary of Product CharacteristicS (SmPC)

14.4.2. Patients HBV positive

Patients with HBV positive can enter the study only after adequate treatment on hepatologist or infection disease specialists. Therapy as per standard care (Indication to HBV therapy as per by EASL criteria 2017) (appendix F)

14.4.3. Patients HCV positive

Anti HCV positive, HCV RNA negative patients can be enrolled without any active measure

14.4.4. Patients HIV positive

Not admitted

14.4.5. Contraception

Non-sterilized female patients of reproductive age group and male patients should use effective methods of contraception through defined periods during and after study treatment as specified below.
Female patients must meet 1 of the following:

- Postmenopausal for at least 1 year before the screening visit, or surgically sterile, or
- If they are of childbearing potential, agree to practice 2 effective methods of contraception from the time of signing of the informed consent form through 6 months after the last dose of study drug (since Deferasirox can reduce the effectiveness of hormonal contraceptives, it is recommended that women of childbearing age use additional or alternative non-hormonal contraceptice methods), or
- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, post ovulation methods] and withdrawal are not acceptable methods of contraception.)

14.5. Excluded/admitted concomitant medication.

Admitted concomitant medication: as per clinical practice. Concomitant rHuEpo is admitted and registered (patients who receive rHuEpo during the year study will not be evaluated for erythroid response). No additional experimental therapy is admitted.
A standard pre-transfusion Hemoglobin level will be indicated (Hb threshold for transfusion Hb 9 g/dl or 10 g/dl for patients with cardiac disease) to be maintained during the study year. Pre transfusion hemoglobin level and transfusions date and frequencies will be recorded.
A transfusional ratio will be determined: number of units received in a predetermined period/ mean pre-transfusion Hb level.

14.6. Toxicity

14.6.1. Hematological and non hematological toxicity

A summary of the safety profile of DFX-FCT is available in the approved SmPC (section 4.8 approved label)

14.7. (Drug) dose modification

A summary of the information and specials warnings and precautions for use as per clinical practice is available the approved SmPC section 4.4 approved label)

Table 1. (Drug) dose modification

NCI CTCAE Toxicity Grade	ACTION REQUIRED
Grade III-IV	Drug deferral by PI judgment
Serum ferritin < 300 ng/ml	DFX deferral

Table 2: Dose modification steps for patients who need DFX dose modifications during the study

Planned dose	Modified dose
DFX FCT 3.5 mg/kg/day	No dose modification allowed.

14.8. Toxicities management

As per approved label (Summary of Product Characteristic (SmPC)

14.9. Rescue Medication

This study propones low doses of well-known drug. Recent studies clearly demonstrated safety of Deferasirox even in patients with low ferritin level receiving standard Deferasirox doses. Criteria for premature protocol termination for toxicity of lack of efficacy has been above specify. No specific rescue medication is applicable.

14.10. Benefit/Risk assessment

Because of the low doses, the ability to directly measure toxic iron species and the potential to remove them from circulation, the protocol tries to define the minimum effective dose of iron chelating therapy. Therefore, the risk/benefit ratio appears clearly in favor of benefit (iron toxicity and iron overload prevention, drug toxicity prevention).

15. REMOVAL OF SUBJECTS FROM TREATMENT AND/OR STUDY

15.1. Discontinuation from study treatment

Patients may voluntarily discontinue from the study treatment for any reason at any time.
If a patient decides to discontinue from the study treatment, the investigator should make a reasonable effort (e.g. telephone, e-mail, letter) to understand the reason for this decision and record this information in the patient's chart and on the appropriate CRF pages.
Patients may be considered withdrawn if they state an intention to withdraw, fail to return for visits / lost to follow-up for any other reason.
The investigator should discontinue study treatment for a given patient if, he/she believes that continuation would be detrimental to the patient's well-being.

Study treatment must be discontinued under the following circumstances:

- Death
- Adverse Events
- Abnormal laboratory value
- Pregnancy
- Unsatisfactory therapeutic effect
    - patients with evident inadequate chelation (Transfusion burden > 4 units/month over a three consecutive months period and > 1 PRBC unit/week and a total number of PRBC received >20) the protocol will be discontinued and the patient will be treated as clinically (by label) indicated
- Unwillingness to comply with study procedures/treatment as per protocol
- Discovery of patient ineligibility
- Intake of prohibited medications
- Protocol violation
- Consent withdrawal
- Lost to follow-up
- Administrative problems
- Premature termination of the study
- In patients with serum ferritin <300 ng/ml therapy will be suspended and the patient will remains in study, with the same transfusion (minimal Hb level 9 g/dl) and study criteria. Patients will restart treatment as soon as serum ferritin arise >300 ng/ml.

15.2. Withdrawal of Consent

Patients are free to withdraw from the study at any time without prejudice to their treatment. When a patient decides to withdraw from the study, she/he should always be contacted in order to obtain information about the reason for withdrawal and to record any adverse events. When possible, the patient should return for a study visit at the time of, or soon after withdrawal, and the relevant assessments should be performed.
If the patient explicitly states his/her wish not to contribute with further data to the study, the assigned FISiM-ETS Study Coordinator should be informed and the withdrawal of consent should be documented by the investigator in the patient's case report form. Information from subsequent ambulatory visits, laboratory or instrumental assessments and any other information on the patient status after consent withdrawal won't be collected in the data base or used for analysis. However, both clinical data collected until patient's withdrawal as well as the data coming from the central review will still be considered as available for the study analysis.

15.3. Patients Lost to Follow up

Every effort will be made to contact patients who fail to return for scheduled visits. A patient is considered lost to follow-up if no information has been obtained when the last patient has completed the clinical phase of the study. During this time site investigator must document attempts to contact the patient either by phone or by letter.

15.4. Premature termination of the study

The sponsor reserves the right to stop the trial at any time. The investigators will be informed of this decision in writing.
The same applies to any investigator willing to discontinue his/her participation to the trial. The investigator must immediately inform the sponsor in writing of this decision.

16. STUDY PROCEDURES TIMEPOINTS

16.1. Screening period (within 3 weeks prior to Study Cycle 1 Day 1)

All subjects will be screened for study eligibility including:

- Medical History;
- Physical Examination;
- ECOG;
- Vital Signs;
- See schedule of assessments

A physical examination will be performed at screening and subsequent study visit. Information about the physical examination must be present in the source documentation at the study site. Significant findings that are present prior to the start of the study drug must be included in the Medical History. Significant findings made after the study drug which meet the definition of an AE must be recorded as Adverse event. All patient will have standing height measured at the screening visit. Body weight and ECOG sate will be recorded at screening and then at every clinic visit

Vital signs determinations are of sitting blood pressure, heart rate, respiratory rate and body temperature and are taken at every visit. All vital sign measurement should be recorded every visit.

- Clinical Laboratory Evaluations;
- 12 lead Electrocardiograms;
- Echocardiogram
- Pregnancy test (if applicable);
- Ophthalmologic examination and audiometry

Patients will undergo auditory and ocular examinations at screening and at end of treatment or at unscheduled visit if needed.
If a patient had an auditory or ophthalmologic exam 6 months prior to the screening and the test results are available, then these tests will not need to be done at screening.
The auditory examination as per clinical practice.
The ophthalmologic examination as per clinical practice
Information about the audiometry and the ocular examinations must be present at the source documentation at the study site. Significant findings made after the study drug which meet the definition of an AE must be recorded as Adverse event.

- A bone marrow aspirate for diagnosis purpose (optional bone marrow biopsy with immune-histochemical and fibrosis evaluation);

- A Marrow aspiration obtained in the six months preceding trial enrollment is considered adequate.
- Peripheral blood samples biological tests;
- NGS (molecular tumor analysis)

16.2. Treatment

According to the label during the first month of treatment renal function (serum creatinine and creatinine clearance) will be checked weekly, liver function (ASL, ALT and serum bilirubin) will be checked every other week).

At any month step and at any transfusional event:

- Physical Examination;
- ECOG;
- Vital Signs;
- Clinical Laboratory Evaluations (see scheduled laboratory examination);
- In addition, at any transfusional event: CBC, Renal function (creatinine and blood urea) and liver function (bilirubin, AST, ALT)
- Peripheral blood immunophenotyping in case of suspicious leukemic dissemination;

16.3. EOT phase

- Physical Examination;
- ECOG;
- Vital Signs;
- Clinical Laboratory Evaluations (see schedule of assessments);
- 12 lead electrocardiograms;
- Echocardiogram
- bone marrow aspirate (if clinically indicated)
- Peripheral blood immunophenotyping in case of suspicious leukemic dissemination;

16.4. Early withdrawn (discontinuation from study treatment)

- Physical Examination;
- ECOG;
- Vital Signs;
- Clinical Laboratory Evaluations;
- Electrocardiograms;
- Peripheral blood / bone marrow immunophenotyping in case of suspicious leukemic dissemination;
- Any test needed if withdrawal is for toxic reason.

16.5. Investigations tests

See schedule of assessments for experimental studies.

Note that sample shipment will be a pre-requisite for study inclusion at some specific time points.

16.6. Schedule of assessments

	Site	Screening	Baseline	Month 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M 9	M 10	M 11	EOS
Informed consent	Local	X													
Inclusion /exclusion criteria	Local	X													
Medical history	Local	X													
Physical examination (including height, weight and BSA)*	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test	Local	if indicated				if indicated						if indicated			
ECOG*	Local	X													
CBC (including reticulocytes)	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Creatinine clearance	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Creatinine	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Urea	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine proteins (measured in non first void sample urine)	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ASL	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ALT	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bilirubin	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Ferritin	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Transferrin saturation	Local	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Erythropoietin	Local	X	X			X			X			X			X
Ophthalmology examination**	Local	X													X
Audiometry**	Local	X													X
12 Lead ECG	Local	X													X
Echocardiography	Local	X													X
Marrow aspiration (WHO 2016 criteria) or biopsy	Local	If clinical indicated													X
Qol Questionnaire	Local		X						X						X
§During the first month of treatment additional laboratory checks must be performed: serum creatinine and calculated creatinine clearance every week, transaminases and serum bilirubin every two weeks (according to SmPC – RCP)															

Concomitant Medications	Recorded from the screening through 30 days after the last dose of treatment
PRBC transfusions	A standard pre-transfusional Hemoglobin level will be indicated (Hb threshold for transfusion Hb 9 g/dl or 10 g/dl for patients with cardiac disease) to be maintained during the study year. Pre transfusional hemoglobin level and transfusions date and frequencies will be recorded. A transfusional ratio will be determined: number of units received in a predetermined period/ Mean pre-transfusional Hb level
Adverse events	Recorded from the first dose of study drug through last patient last visit
Serious Adverse Events	Recorded from signing of the informed consent form through last patient last visit

* A physical examination will be performed at screening and subsequent study visit. Information about the physical examination must be present in the source documentation at the study site. Significant findings that are present prior to the start of the study drug must be included in the Medical History. Significant findings made after the study drug which meet the definition of an AE must be recorded as Adverse event. All patient will have standing height measured at the screening visit. Body weight and ECOG sate will be recorded at screening and then at every clinic visit

Vital signs determinations are of sitting blood pressure, heart rate, respiratory rate and body temperature and are taken at every visit. All vital sign measurement should be recorded for every visit.

** Patients will undergo auditory and ocular examinations at screening and at end of treatment or at unscheduled visit if needed.
If a patient had an auditory or ophthalmologic exam 6 months prior to the screening and the test results are available, then these tests will not need to be done at screening
The auditory examination includes the following assessments: comprehensive audiometry threshold examination, speech recognition
The ophthalmologic examination includes the following assessments: vital acuity test (refraction), tonometry, slit lamp exam of anterior segment, slit lamp exam of the lens, a funduscopic and retinal examination
Information about the audiometry and the ocular examinations must be present at the source documentation at the study site. Significant findings made after the study drug which meet the definition of an AE must be recorded as Adverse event

16.7. Schedule of blood samples assessments for experimental biological study

		Baseline	Month 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M 9	M 10	M 11	EOS
Liver and pancreas MRI (MRI done locally and sent for hepatic and pancreatic iron determination). See Appendix I	Centralized	X												X
NTBI	Centralized	X	X	X	X			X			X			X
LPI	Centralized	X	X	X	X			X			X			X
Epcidine; sTR	Centralized	X			X			X			X			X
Erythroferrone, GDF15, GDF11	Centralized	X			X			X			X			X
MDA Membrane lipid peroxidation	Centralized	X	X	X	X			X			X			X
NGS study	Centralized	X												X

NGS study (molecular tumor analysis) to be performed in case of disease progression

17. EFFICACY MEASUREMENTS AND PARAMETERS

17.1. Efficacy measurement

Patients receiving at least 1 dose of DFX-DT will be considered as Efficacy Population (EP).

17.2. Efficacy parameters

17.2.1. Primary endpoints

	Objective	Endpoint
Primary Objective and endpoints	Balance iron burden in one-year treatment in early phase of transfusion requirement by low dose (3.5 mg/kg) DFX-FCT (prevention of iron overload) as demonstrated by hepatic iron concentration.	Change of hepatic iron from the baseline according to baseline hepatic iron level: For patients with baseline LIC ≤5 mg/g dry weight (dw) ± 1.5 mg/g dw. For patients with baseline LIC >5 mg/g dw ±20% The as demonstrated by R2- MRI (test performed in a 1.5 tesla MRI machine and analyzed following R2 method (75)- Baseline versus EOS. The corresponding secondary efficacy variable will be the absolute change in hepatic iron concentration EOS versus baseline.

17.2.2. Secondary objective and endpoints

	Secondary Objectives	Secondary Endpoints
Descriptive-observational objective	Definition of iron overload (including serological markers and MRI definition of iron loading in different tissues: liver, pancreas), tissue reactive iron species and oxidative stress in MDS at beginning of transfusional history.	Absolute values of serum ferritin, transferrin saturation, NTBI, LPI, liver and pancreas MRI and oxidative stress at baseline.
	Efficacy	Absolute change in hepatic iron concentration EOS versus baseline.
	On year evolution of iron overload serologic markers	Absolute and relative changes in serum ferritin and transferrin

		saturation from baseline to every visit during the whole treatment period.
	Presence and quantitative evolution of toxic serum iron forms (iron tissue reactive species) under low dose DFX therapy.	Proportion of patients with NTBI > normal values and/or LPI > normal values at end of study vs baseline. Changes in NTBI and LPI from baseline to every visit during the whole treatment period.
	Verify if regular suppression of the “free iron forms” prevent accumulation of tissue iron.	Relationship between NTBI and LPI with serum ferritin and liver and pancreas iron overload (MRI) at end of study versus baseline
	Evaluate the overall safety of deferasirox FCT formulation in patients with lower risk MDS at the beginning of their transfusional history	Overall safety, as measured by frequency and severity of reported AEs and SAEs and changes in laboratory values from baseline: serum creatinine, creatinine clearance ALT, AST, complete blood count (platelets, RBC and WBC) and total direct and indirect bilirubin).
	Leukemic transformation (progression to leukemia or higher rIPSS scores)	Proportion of patients with a disease progression (progression defined as a transition into a higher MDS risk group based on revised IPSS scoring or progression to AML) Time to progression (defined as above) or to leukemia transformation.
	Hemopoietic response	Percentage of patients with hematologic improvements in term of erythroid response following IWG 2006 criteria. Time to reach transfusion dependence defined as > 2 PRBC units/months for 3 months for patients with pre-transfusional Hb < 9.0 g/dl (or <10 g/dl for patients with cardiac disease) or a total number of PRBC units received = 20 from baseline. Absolute reticulocytes count from baseline to every visit during the whole treatment period. Absolute values and evolution of serum transferrin receptor, GDF11, GDF 15 and erythroferrone from baseline to every visit during the whole treatment period. Evaluation of transfusional ratio: number of units received in a

		predetermined period/ Mean pre-transfusional Hb level. Compared with other studies on general population (study and FISiM-ETS registry). Patients receiving concurrent rHuEpo will not be considered for erythroid response
	Costs analysis	Cost and outcome with low dose in early chelation vs. chelation treatment in MDS patients with transfusional iron overload in accordance with either local or international guidelines
Exploratory objective and endpoint	Study of biological cellular damage by iron toxicity before and during treatment	MDA values during the study and relationship to NTBI and LPI values

18. SAFETY MONITORING AND REPORTING

Safety monitoring will be based on the i) evaluation of changes from baseline throughout the whole study in physical examination, heart rate/rhythm, blood pressure, renal function, liver function and laboratory findings, ii) a global assessment of organ function; and iii) the observation and report of any adverse event (AE).

18.1. Adverse Events

An adverse event is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) that occur after patient's signed informed consent has been obtained.
Once the study ICF is signed, all AEs will be captured in the Adverse Event CRF.
The severity of an AE will be assessed on the basis of the NCI CTCAE version 4.03 (please see forreference:https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_ 8.5x11.pdf.).

All non-Serious Adverse Events, all reports of drug exposure during pregnancy, all reports of misuse and abuse of the drug, all reports describing the occurrence of other "special scenarios" (including reports of drug-drug/drug-food interaction, drug use during lactation or breast-feeding, lack of effectiveness, overdose, drug maladministration or accidental exposure, dispensing errors/medication errors, withdrawal or rebound symptoms, irrespective of whether a clinical event has occurred) and all SAEs will be collected into the patients case report form. The same will be done for any other information that may suggest a change in the benefit-risk profile for the drug.
Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. Adverse event monitoring should be continued for at least 30 days following the last dose of study treatment. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event. AEs will not be collected beyond 30 days after the last dose of study treatment. The occurrence of adverse events should be sought by non-directive questioning of the patient (subject) during the screening process after signing informed consent and at each visit during the study. Adverse events also may be detected when they are volunteered by the patient (subject) during the screening process or between visits, or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

- The severity grade (CTCAE Grade 1-5)
- Its duration (Start and end dates)
- Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes)
- Action taken with respect to study treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable).
- Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
- Whether it is serious, where a serious adverse event (SAE) is defined as in Section 18.2 and which seriousness criteria have been met.
- Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequalae, fatal, unknown)

If the event worsens the event should be reported a second time in the CRF noting the start date when the event worsens in toxicity. For grade 3 and 4 adverse events only, if improvement to a lower grade is determined a new entry for this event should be reported in the CRF noting the start date when the event improved from having been Grade 3 or Grade 4.

All adverse events should be treated appropriately. If a concomitant medication or non- drug therapy is given, this action should be recorded on the Adverse Event CRF.

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

18.2. Serious Adverse Events

18.2.1 Definitions

Serious adverse event (SAE) is defined as one of the following:

- Is fatal or life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Note that hospitalizations for the following reasons should not be reported as serious adverse events: Routine treatment or monitoring of the study indication
  - Progression/relapse of underlying disease (including fatal outcomes)
  - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
  - Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event.
-

18.2.2 Reporting

For patients who sign the Screening ICF, SAE collection will start upon signing the Screening ICF. SAEs will only be reported if the event is suspected to be causally related to a study procedure as assessed by the investigator (e.g. an invasive procedure such as biopsy). SAEs will be followed until resolution or until clinically relevant improvement or stabilization. If the Study ICF is not signed (screen failure), SAE collection ends 30 days after the last study related procedure.

For patients who sign the Study ICF, SAE collection starts at time of Study informed consent whether the patient is a screen failure or not.

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided Study informed consent and until at least 30 days after the patient has stopped study treatment must be reported to Safety Responsible within 24 hours of learning of its occurrence. Any additional information for the SAE including complications, progression of the initial SAE, and recurrent episodes must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Any SAEs experienced after the 30 day safety evaluation follow-up should only be reported to Safety Responsible if the investigator suspects a causal relationship to the study treatment.

Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form; all applicable sections of the form must be completed in order to provide a clinically thorough report. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and submit the completed form within 24 hours to the Safety Responsible of the study. Detailed instructions regarding the SAE submission process and requirements for signatures are to be found in the investigator folder provided to each site.

Follow-up information is submitted in the same way as the original SAE Report. Each re- occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the patient continued or withdrew from study participation.

If the SAE is not previously documented and is thought to be related to the study treatment, the Safety Responsible of the study and the Novartis safety desk may urgently require further information from the investigator for Health Authority reporting. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

All Collected SAEs in patients exposed to the Novartis drug and SUSARs should be transferred to Novartis safety desk within 15 calendar days of awareness.

The FISiM-ETS Pharmacovigilance will provide Novartis with a copy of the development safety update report (DSUR) at the time of submission to the Regulatory Authority and Ethics Committees.

CONTACT DETAILS FOR PHARMACOVIGILANCE

Daniela Gioia

☎: +39 0131.033156

Fax: +39 0131 263455

e-mail:drugvigilance@filinf.it

19. PREGNANCIES

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to the Safety Responsible of the study within 24 hours of learning of its occurrence. Occurrence of Pregnancy should be monitored for 90 days after stopping treatment (i.e. End of treatment or Cross-Over End of treatment whichever comes last). The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. The follow- up to three months after delivery is mandatory for all reported pregnancy cases and in cases of live birth, the follow-up on any development issues or abnormality that would not be seen at birth.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the Safety Responsible of the study within 24 hours from its learning. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

Pregnancy and its follow-up should be also reported to Novartis safety desk within 15 calendar days of awareness.

e-mail:drugvigilance@filinf.it
Fax number: +39 0131 263455

20. STATISTICAL CONSIDERATIONS

20.1. Study design

One year low dose (3,5 mg/kg/day DFX-FCT) in patients at the beginning of their transfusion history. Dose activity will be monitored by regular NTBI /LPI suppression and by prevention of iron accumulation (hepatic iron concentration and other tissue and serologic parameters) comparing baseline versus end of study iron concentration.

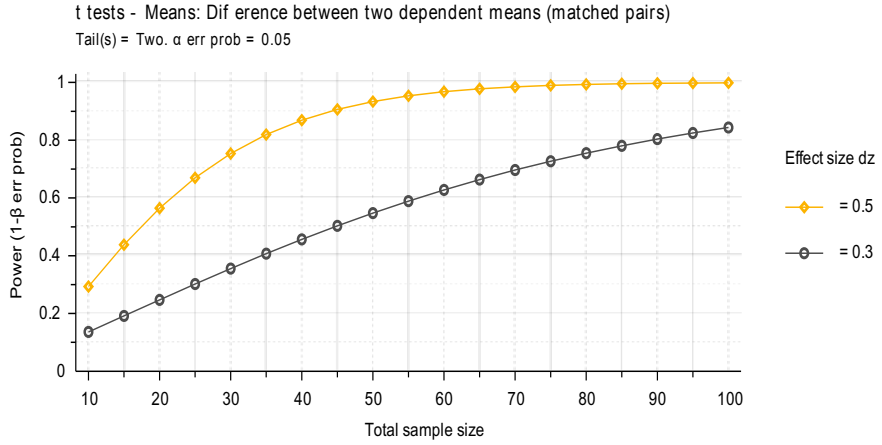
A standard pre-transfusional hemoglobin level will be indicated (Hb threshold for transfusion Hb 9 g/dl or 10 g/dl for patients with cardiac disease) to be maintained during the study year. Pre transfusional hemoglobin level and transfusions date and frequencies will be recorded. A transfusional ratio will be determined: number of units received in a predetermined period/ mean pre-transfusional Hb level.

Open label, single arm, fixed dose phase II study.

20.2. Sample size calculation

Including 50 subjects in the study, we can reach almost 60% power to detect small effect size 0.30 (with p value of 0.05) whereas, we have almost 100% to detect moderate effect size of 0.50 (with p-value of 0.05). On the basis of previous experience, the low dosage, the early intervention and the FCT preparation a 20% drop out rate is expected (10 additional patients to be enrolled)

Methods:



The power test was performed by G power 3.1 software by using the t test Means: Difference between two dependent means (matched pairs).

As demonstrated by pour sample size analyses, we have enough power to detect an effect size between 0.3 and 0.5 considering a potential error of 0.05, which is largely accepted from scientific community.

The effect size is calculated on a common scale by G-power program which allows to compare the effectiveness of different variables on the same outcome. In other words, quantifies the magnitude of the difference of a variable between two population groups.

To interpret the resulting number, most scientists use this general guide developed by Cohen:

- < 0.1 = trivial effect
- 0.1 - 0.3 = small effect
- 0.3 - 0.5 = moderate effect
- > 0.5 = large difference effect

20.3. Statistical method and data analyses.

Analysis population: the planned enrolment target is 50 patients (+ 20% drop out rate) for a total number of 60 patients.

Data from all centers participating in this study will be pooled for analyses.
Standard descriptive analyses will include:
- Frequencies and percentages for categorical data;
- n, mean, standard deviation, minimum, median, 25th and 75th percentiles and maximum for continuous data.

Whenever possible, the analysis will be performed by visit.

Analysis sets

Full Analysis Set (FAS): All enrolled patients.

Safety analysis set (SS): All enrolled patients who will have received at least one dose of study drug and have at least one post-baseline safety assessment. Note that an entry on the Adverse Event CRF of a statement that a patient had no adverse events constitutes a safety assessment.

Per Protocol analysis set (PP): All enrolled patients who will have received at least one dose of study drug without any major protocol violations and complete the treatment study phase or withdraw from the study due to unsatisfactory therapeutic effect.

Patient demographics/other baseline characteristics
Demographic and other baseline data (including disease characteristics and transfusion history, etc) will be summarized descriptively for the Safety analysis set by means of the statistics above mentioned. Summary tables will be provided about medical history coded by System Organ Class and Preferred Term of MedDRA dictionary.

Treatments (study treatment, concomitant therapies,compliance)
The descriptive analysis listed below will be reported for the safety analysis set.

Study drug
The duration of study drug exposure, as well as average prescribed doses, will be summarized. Any dose adjustments and reasons that led to the adjustments will be listed. The number and duration of dose interruptions will also be described.

Compliance
Compliance based on prescribed amount of study medication versus amount of medication taken based on dispensed and returned amount of study medication will be summarized.

Concomitant Therapies
Concomitant medications and significant non-drug therapies prior to and after the start of the study treatment will be coded by WHO Drug Reference List dictionary and frequencies will be calculated according to drug classes.

The primary objective is prevention of iron accumulation measured by (primary variable) Change of hepatic iron from baseline according to baseline hepatic iron level: Baseline versus end of study.
For patients with baseline LIC ≤5 mg/g dry weight (dw) ± 1.5 mg/g dw.
For patients with baseline LIC >5 mg/g dw ±20% (by R2- MRI ; test performed in a 1.5 tesla MRI machine and analyzed following R2 method (75).
The corresponding secondary efficacy variable will be the absolute change in hepatic iron concentration EOS versus baseline.

Difference between baseline versus EOT will be measured as continuous variables and analyzed with parametric or non parametric statistical tests depending by normality of distribution.

Secondary endpoint:

Observational: demographics and other baseline characteristics will be study with descriptive statistics. Analysis will be done on FAS and PP.

As the same way secondary endpoints will be evaluated as continuous or nominal variables and analyzed accordingly with parametric or non parametric tests. Efficacy parameters analysis will be done on FAS and PP.

Final Safety parameters will be measured in full analyses set and in all patients who received at least one dose of study drug (safety set).

21. INDEPENDENT DATA SAFETY MONITORING COMMITTEE (DMSC)

The FISiM-ETS on its own initiative and responsibility set up an independent external DSMC. The DSMC consist in experts independent from the sponsor.
The aim of the DSMC is to assess, at intervals during the course of the trial, the progress of the trial, the trial safety data and the trial outcome data with a view to recommending whether the trial should continue, be modified or be terminated.

The DSMC will be composed by:
1. Two independent scientists with expertise in the treatment of iron overload and or MDS
2. The biostatistician of the study
3. An independent statistician with expertise in the methodology of clinical trials and data analysis.

Roles of DSMC (suggested, not exclusive):
1. To review ongoing safety data throughout the study.
2. To monitor evidence for treatment benefit and thus decide when/whether the main trial question has been answered.
3. To monitor evidence for treatment harm (toxicity).
4. To decide whether to recommend changes to the protocol.
5. To decide whether to recommend that the trial continues to recruit participants or whether recruitment should be terminated.
6. To review final analysis of the data.
7. To discuss final data with PI and the sponsor.

Planned meetings:
1. A minimum of two call conferences that include preparation, working time during the meeting, and writing reports.

22. GOOD CLINICAL PRACTICE, QUALITY CONTROL AND QUALITY ASSURANCE

22.1. Monitoring, Audits and Inspections

During the study the monitoring will be prevalently made by e-mail and telephone. The field monitor will visit the site, when needed, mainly in presence of data incongruity, to check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the protocol and to Good Clinical Practice and the progress of enrolment. Key study personnel must be available to assist the field monitor during these visits.
The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information on CRFs must be traceable to these source documents in the patient's file. The investigator must also keep the original informed consent form signed by the patient (a signed copy is given to the patient).
The investigator must give the monitor access to all relevant source documents to confirm their consistency with the CRF entries. FISiM-ETS Safety Monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of data that will be used for all primary and safety variables. Additional checks of the consistency of the source data with the CRFs are performed according to the study-specific monitoring plan. No information in source documents about the identity of the patients will be disclosed.

22.2. Investigator(s) responsibilities

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice. The investigator must give the monitor access to relevant records to confirm the above.
The Investigator(s) is responsible for keeping a record of all patients who sign an Informed Consent Form and are screened for entry into the study. For those patients who fail screening the reason(s) for exclusion must be recorded in the patient's source documents.
No procedure/assessment/measurement/test other than those outlined here, or in the schedule of study assessments, is to be performed without the prior written approval of Principal Investigator, or unless deemed by the investigator(s) as necessary for the patient's medical care. Investigator(s) and/or

authorized designee(s) must enter study data onto electronic CRFs supplied by FISiM-ETS. The data on the CRF will be recorded in an anonymous manner to protect the patient's identity by using a unique identifier that will prevent personal identifiable information.
The Investigator(s), or a designated member of the Investigators' staff, must be available at some time during monitoring visits to review data and resolve any queries and to allow direct access to the patient's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification. The CRFs must be completed as soon as possible after the patient's visit, but no later than prior to each monitoring visit and be made available to the FISiM-ETS representative(s) so that the accuracy and completeness may be checked.

23. ETHICAL AND REGULATORY STANDARDS

23.1. Institutional Review Board/Independent Ethics Committee Review Approval

This study will be conducted according to the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Patients (see: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ for more information). The review of this protocol by the IRB/IEC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Patients and Part 56 Institutional Review Boards. Before implementing this study, the protocol, the proposed informed consent form and other information to patients, must be reviewed by a properly constituted IRB/IEC. A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC must be given to FISiM-ETS before the study initiation. The names and occupations of the chairman and the members of the IRB/IEC must be supplied to FISiM-ETS.
The FISiM-ETS as sponsor of the study, together with site Investigator(s), will be responsible for preparing documents, where ever applicable, for submission to the relevant IRB/IEC and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study.
A copy of the IRB/IEC approval for the protocol and the Informed Consent is to be provided to FISiM and site Investigator(s). The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number.
The Investigator(s) is responsible for notifying the FISiM Safety Monitoring Office and the IRB/IEC of any serious deviations from the protocol, or anything else that may involve added risk to patients.
Any advertisements used to recruit patients for the study must be reviewed and approved by FISiM and the IRB/IEC prior to use.
Before the start of the study, the FISiM-ETS will provide the IRB/IEC with current and complete copies of the following documents:

1. final protocol and, if applicable, amendments
2. informed consent form (and any other written materials to be provided to the subjects)
3. Currently approved SmPC
4. information on compensation for study-related injuries
5. investigator's curriculum vitae or equivalent information (unless not required, as documented by IRB/IEC)
6. any other documents that the IRB/IEC requests to fulfill its obligation.

During the study the FISiM-ETS according with site investigators will send the following documents to the IRB/IEC for their review and approval, where appropriate:

1. protocol amendments
2. revision(s) to informed consent form and any other written materials to be provided to subjects
3. revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
4. currently approved SmPC-summaries of the status of the study (at least annually or at intervals stipulated in guidelines of the IRB/IEC)
5. reports of adverse events that are serious, unexpected and associated with the investigational drug
6. new information that may affect adversely the safety of the subjects or the conduct of the study

7. deviations from or changes to the protocol to eliminate immediate hazards to the subjects
8. report of deaths of subjects under the investigator's care
9. notification if a new investigator is responsible for the study at the site
10. any other requirements of the IRB/IEC

23.2. Protocol Amendments Approval

Any amendment to this protocol that seems appropriate, as the study progresses will be submitted to the IRB/IEC for written approval before the implementation of the amended version. The written signed approval from the IRB/IEC should refer specifically to the investigator(s) and to the protocol number and title and mention any amendment numbers that are applicable. Amendments that are administrative in nature do not require IRB/IEC approval but will be submitted to the IRB/IEC for information purposes.

24. ADMINISTRATIVE PROCEDURES

24.1. Curriculum vitae

An updated copy of the curriculum vitae of each investigator and sub-investigator will be provided to the FISiM-ETS Start Up prior to the beginning of the study.

24.2. Confidentiality agreement

All goods, materials, information (oral or written) and unpublished documentation provided to the investigators (or any company acting on their behalf), inclusive of this study, the patient case report forms are the exclusive property of FISiM-ETS.
They may not be given or disclosed by the investigator or by any person within his authority either in part or in totality to any unauthorized person without the prior written formal consent of FISiM-ETS.
It is specified that the submission of this study and other necessary documentation to the Ethics Review Committee or a like body is expressly permitted, the Ethics Committee members having the same obligation of confidentiality.
The investigator shall consider as confidential and shall take all necessary measures to ensure that there is no breach of confidentiality in respect of all information accumulated, acquired or deduced in the course of the trial, other than that information to be disclosed by law.

24.3. Record retention in investigating centers.

The investigator must maintain all study records, patient files and other source data for the maximum period of time permitted by the hospital, institution or private practice.
However national regulations should be taken into account, the longest time having to be considered.
For trials performed in the European Community, the investigator is required to arrange for the retention of the patient identification codes for at least 15 years after the completion or discontinuation of the trial.
Any center will notify the sponsor before destroying any data or records.

24.4. Ownership of data and use of the study results.

The sponsor has the ownership of all data and results collected during this study. In consequence the sponsor reserves the right to use the data of the present study, either in the form of case report forms (or copies of these), or in the form of a report, with or without comments and with or without analysis.

24.5. Authorship

The first results of the trial will be published after complete data collection and evaluation of the primary endpoint. Partial or preliminary results can be published beforehand. Publication is to be initiated by the chairmen in charge of the study with approval of coordinators.
Any publication in the form of a lecture, poster or article must be prospectively approved by the Scientific Committee of FISiM-ETS.
The authors will be proposed (according to the updated FISiM-ETS publication rules) by the chairmen in charge of the study, approved by coordinators following recruitment criteria and scientific contribution.
All study data and publications are the property of the FISiM-ETS.

The target publication plan will include:

- ASH 2019 abstract: Study presentation and enrolment status
- SIE 2019 encore abstract: Study presentation and enrolment status
- EHA 2020 abstract: baseline study population
- ASH 2020 encore abstract: baseline study population
- ASH 2021 abstract: primary data

24.6. Insurance coverage

The Investigator-sponsor of the Study must ensure that adequate insurance coverage is available to the patients, in accordance with the ICH Guidelines of Good Clinical Practice. Such coverage must extend to all damages deriving from the study, to the Protocol Study exclusion of those attributable to willful misconduct or negligence of the institution or investigator. A copy, or excerpt, or insurer's certificate, attesting the existence and amount of such coverage at least for the duration of the study must be supplied as part of the study documentation to the review and approval of the IEC.
A specific insurance with company [HDI Gerling number 390-01583564-30010) has been concluded for patients enrolled in this study. No extra expenses, neither for therapies nor for clinical or laboratory procedures can be asked or expected to be paid by SSN or patients.

24.7. Protocol amendments procedures

It is specified that the appendices attached to this study and referred to in the main text of this study, form an integral part of the study.
No changes or amendments to this study may be made by the investigator or by the sponsor after the study has been agreed to and signed by both parties unless such change(s) or amendment(s) have been fully discussed and agreed upon by the investigator and the FISiM-ETS.
Any change agreed upon will be recorded in writing, the written amendment will be signed by the investigator and by the sponsor and the signed amendment will be appended to this study.
Approval / advice of amendments by Ethics Review Committee and Competent Authorities are required prior to their implementation, unless there are overriding safety reasons.
If the change or deviation increases risk to the study population, or adversely affects the validity of the clinical investigation or the subject's rights, full approval / advice must be obtained prior to implementation. For changes that do not involve increased risk or affect the validity of the investigation or the subject's rights, approval / advice may be obtained by expedited review, where applicable.
In some instances, an amendment may require a change to a consent form. The investigator must receive approval / advice of the revised consent form prior to implementation of the change. In addition, changes to the case report forms, if required, will be incorporated in the amendment.

25. DATA HANDLING AND RECORD KEEPING

25.1. Data/documents

The investigator(s) must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug, that is copies of CRFs and source documents original documents, data, and records (e.g., hospital records; clinical and office charts; laboratory notes; memoranda; patient's diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiches; photographic negatives, microfilm, or magnetic media; x-rays; patient files) and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study are complete, accurate, filed and retained.

25.2. Data Management

Data will be entered into the clinical database as per FISiM-ETS SOPs. These data will be electronically verified through use of on-line checks during data entry, and through programmed edit checks specified by the clinical team. Discrepancies in the data will be brought to the attention of the clinical team, and investigational site personnel, if necessary, in the form of a Data Clarification Form (DCF). Resolutions

to these issues will be reflected in the database. An audit trail within the system will track all changes made to the data.

25.3. Retention of Records

The investigator(s) must maintain records of all study documents and supporting information relating to the conduct of the study. This documentation includes, but is not limited to, protocols, case report forms, advertising for patient participation, adverse event reports, patient source data, correspondence with health authorities and IRBs/IECs, informed consent forms, investigator(s) curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. Patient files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice specified below. The study monitor must be consulted if the investigator(s) wishes to assign the study files to someone else, remove them to another location or is unable to retain them for a specified period. The investigator(s) must retain study records for the time period according to local laws or requirements, whichever is longer. The monitor will inform the investigator(s) of the dates for retention. All study documents should be made available if required by relevant health authorities. The investigator(s) records must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by other applicable regulatory requirements.

26. PRIVACY OF PERSONAL DATA

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational product(s) used in this study. These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.
The investigator-sponsor ensures that the personal data will be:

1. processed fairly and lawfully
2. collected for specified, explicit, and legitimate purposes and not further processed in a way incompatible with these purposes
3. adequate, relevant, and not excessive in relation to said purposes
4. accurate and, where necessary, kept current

Explicit consent for the processing of personal data will be obtained from the participating subject (or his/her legally acceptable representative) before collection of data. Such consent should also address the transfer of the data to other entities and to other countries. The subject has the right to request through the investigator access to his/her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps should be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.
Patients will be registered in the study via web site the end of their staging, before beginning the treatment. The name of the patient will not be asked for not recorded at the Data Center. A sequential identification number will be automatically attributed to each patient registered in the trial. This number will identify and must be included on all case report form.

27. REFERENCES

1. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. Am Soc Hematology; 1997;89(6):2079–88.

2. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sol?? F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454–65.

3. Cazzola M, Della Porta MG, Malcovati L. Clinical relevance of anemia and transfusion iron overload in myelodysplastic syndromes. Hematology Am Soc Hematol Educ Program. 2008;166–75.

4. Malcovati L. Impact of transfusion dependency and secondary iron overload on the survival of patients with myelodysplastic syndromes. Leuk Res [Internet]. Elsevier; 2016 Dec 11;31:S2–6. Available from: http://dx.doi.org/10.1016/S0145-2126(07)70459-9

5. Malcovati L, Della Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: A basis for clinical decision making. J Clin Oncol. 2005;23(30):7594–603.

6. de Swart L, Smith A, Fenaux P, Sanz G, Hellstrom-Lindberg E, Symeonidis A, et al. Early Mortality in 1000 Newly Diagnosed MDS Patients with Low- and Intermediate-1 Risk MDS in the European Leukemianet MDS (EUMDS) Registry. Blood [Internet]. 2015 Oct 26;120(21):3830 LP-3830. Available from: http://www.bloodjournal.org/content/120/21/3830.abstract

7. Malcovati L, Hellström-Lindberg E, Bowen D, Adès L, Cermak J, Del Cañizo C, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: Recommendations from the European LeukemiaNet. Blood. 2013. p. 2943–64.

8. Cui R, Gale RP, Zhu G, Xu Z, Qin T, Zhang Y, et al. Serum iron metabolism and erythropoiesis in patients with myelodysplastic syndrome not receiving RBC transfusions. Leuk Res. England; 2014 May;38(5):545–50.

9. Angelucci E, Barosi G, Camaschella C, Cappellini MD, Cazzola M, Galanello R, et al. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. Haematologica. 2008;93:741–52.

10. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, et al. Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood [Internet]. 2007;109(10):4586–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1885508&tool=pmcentrez&rendertype=abstract

11. Park S, Sapena R, Kelaidi C, Vassilieff D, Bordessoule D, Stamatoullas A, et al. Ferritin level at diagnosis is not correlated with poorer survival in non RBC transfusion dependent lower risk de novo MDS. Leuk Res. England; 2011 Nov;35(11):1530–3.

12. Chee CE, Steensma DP, Wu W, Hanson CA, Tefferi A. Neither serum ferritin nor the number of red blood cell transfusions affect overall survival in refractory anemia with ringed sideroblasts. Am J Hematol. 2008;83(8):611–3.

13. Cermak J, Kacirkova P, Mikulenkova D, Michalova K. Impact of transfusion dependency on survival in patients with early myelodysplastic syndrome without excess of blasts. Leuk Res. England; 2009 Nov;33(11):1469–74.

14. Li B, Xu Z, Gale RP, Qin T, Zhang Y, Xiao Z. Serum ferritin is an independent prognostic factor in Chinese with myelodysplastic syndromes classified as IPSS Intermediate-1. Acta

Haematol. Switzerland; 2013;129(4):243–50.

15. Kikuchi S, Kobune M, Iyama S, Sato T, Murase K, Kawano Y, et al. Prognostic significance of serum ferritin level at diagnosis in myelodysplastic syndrome. Int J Hematol. Japan; 2012 May;95(5):527–34.

16. Komrokji RS, Corrales-Yepez M, Kharfan-Dabaja MA, Al Ali NH, Padron E, Rollison DE, et al. Hypoalbuminemia is an independent prognostic factor for overall survival in myelodysplastic syndromes. Am J Hematol. United States; 2012 Nov;87(11):1006–9.

17. Pileggi C, Di Sanzo M, Mascaro V, Marafioti MG, Costanzo FS, Pavia M. Role of serum ferritin level on overall survival in patients with myelodysplastic syndromes: Results of a meta-analysis of observational studies. PLoS One. United States; 2017;12(6):e0179016.

18. Angelucci E, Urru SAM, Pilo F, Piperno A. Myelodysplastic Syndromes and Iron Chelation Therapy. Mediterrean J Hematol Infect Dis. 2017;1–10.

19. Pilo F, Di Tucci AA, Dessalvi P, Caddori A, Angelucci E. The evolving clinical scenario of myelodysplastic syndrome: The need for a complete and up to date upfront diagnostic assessment. European Journal of Internal Medicine. 2010. p. 490–5.

20. Gu S, Xv Y, Fei C, Xiao C, Guo J, Zhao Y, et al. Labile plasma iron, more practical and more sensitive to iron overload in myelodysplastic syndromes. Hematology. England; 2017 Jan;22(1):9–15.

21. Ivars D, Orero MT, Javier K, Diaz-Vico L, Garcia-Gimenez JL, Mena S, et al. Oxidative imbalance in low/intermediate-1-risk myelodysplastic syndrome patients: The influence of iron overload. Clin Biochem. United States; 2017 Nov;50(16–17):911–7.

22. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. Free Radic Biol Med [Internet]. Elsevier; 2014;72:23–40. Available from: http://dx.doi.org/10.1016/j.freeradbiomed.2014.03.039

23. de Swart L, Reiniers C, Bagguley T, van Marrewijk C, Bowen D, Hellström-Lindberg E, et al. Labile plasma iron levels predict survival in patients with lower-risk Myelodysplastic syndromes. Haematologica [Internet]. 2017 Nov 9; Available from: http://www.haematologica.org/content/early/2017/11/06/haematol.2017.171884.abstract

24. Chang C, Gu S, Guo J, Zhao Y, Fei C, Li X. Labile plasma iron (LPI), more practical and more sensitive to iron overload in myelodysplastic syndromes. Blood [Internet]. Taylor & Francis; 2015;126(23):5230. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L72175920%5Cnhttp://www.bloodjournal.org/content/126/23/5230%5Cnhttp://rug.on.worldcat.org/atoztitles/link/?sid=EMBASE&issn=00064971&id=doi:&atitle=Labile+plasma+iron+%28LPI%29%2C+mo

25. de Swart L, Hendriks JCM, van der Vorm LN, Cabantchik ZI, Evans PJ, Hod EA, et al. Second international round robin for the quantification of serum non-transferrin-bound iron and labile plasma iron in patients with iron-overload disorders. Haematologica. Italy; 2016 Jan;101(1):38–45.

26. Hershko C, Graham G, Bates GW, Rachmilewitz EA. Non-specific serum iron in thalassaemia: an abnormal serum iron fraction of potential toxicity. Br J Haematol. England; 1978 Oct;40(2):255–63.

27. Hershko C, Link G, Cabantchik I. Pathophysiology of iron overload. Ann N Y Acad Sci. United States; 1998 Jun;850:191–201.

28. Esposito BP, Breuer W, Sirankapracha P, Pootrakul P, Hershko C, Cabantchik ZI. Labile plasma iron in iron overload: Redox activity and susceptibility to chelation. Blood. 2003;102(7):2670–7.

29. Santini V, Alessandrino PE, Angelucci E, Barosi G, Billio A, Di Maio M, et al. Clinical management of myelodysplastic syndromes: Update of SIE, SIES, GITMO practice guidelines. Leuk Res. 2010;34(12):1576–88.

30. Gattermann N. Overview of guidelines on iron chelation therapy in patients with myelodysplastic syndromes and transfusional iron overload. International Journal of Hematology. 2008. p. 24–9.

31. Dessì C, Leoni G, Moi P, Danjou F, Follesa I, Foschini ML, et al. Thalassemia major between liver and heart: Where we are now. Blood Cells Mol Dis [Internet]. 2015;55(1):82–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25976473

32. Taher AT, Porter JB, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, et al. Approaching low liver iron burden in chelated patients with non-transfusion-dependent thalassemia: the safety profile of deferasirox. Eur J Haematol. England; 2014 Jun;92(6):521–6.

33. Gattermann N, Coll R, Jacobasch L, Allameddine A, DeBonnett L, Azmon A, et al. Effect of Deferasirox Combined with Erythropoietin Versus Erythropoietin Alone on Erythroid Response in Patients with Low- and Int-1-Risk Myelodysplastic Syndromes: Results of the Phase II Kallisto Trial. Blood [Internet]. 2017 Dec 7;130(Suppl 1):2984 LP-2984. Available from: http://www.bloodjournal.org/content/130/Suppl_1/2984.abstract

34. Coates TD, Carson S, Wood JC, Berdoukas V. Management of iron overload in hemoglobinopathies: What is the appropriate target iron level? Ann N Y Acad Sci. 2016;1368(1):95–106.

35. Ghugre NR, Gonzalez-Gomez I, Butensky E, Noetzli L, Fischer R, Williams R, et al. Patterns of hepatic iron distribution in patients with chronically transfused thalassemia and sickle cell disease. Am J Hematol. 2009;84(8):480–3.

36. Kakhlon O, Cabantchik ZI. The labile iron pool: characterization, measurement, and participation in cellular processes(1). Free Radic Biol Med. United States; 2002 Oct;33(8):1037–46.

37. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol [Internet]. 2005;18(2):277–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15737890

38. Breuer W, Hershko C, Cabantchik ZI. The importance of non-transferrin bound iron in disorders of iron metabolism. Transfus Sci. England; 2000 Dec;23(3):185–92.

39. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med [Internet]. 2000;343:327–31. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10922422

40. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology [Internet]. 2011;283(2–3):65–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21414382

41. Porter JB, Walter PB, Neumayr LD, Evans P, Bansal S, Garbowski M, et al. Mechanisms of plasma non-transferrin bound iron generation: Insights from comparing transfused diamond blackfan anaemia with sickle cell and thalassaemia patients. Br J Haematol. 2014;167(5).

42. Porter JB, Garbowski M. The pathophysiology of transfusional iron overload. Hematol Oncol Clin North Am [Internet]. 2014;28(4):683–701. Available from: http://dx.doi.org/10.1016/j.hoc.2014.04.003

43. Angelucci E, Pilo F, Coates TD. Transplantation in thalassemia: Revisiting the Pesaro risk

factors 25 years later. Am J Hematol [Internet]. 2017 Feb 1;n/a-n/a. Available from: http://dx.doi.org/10.1002/ajh.24674

44. Vinchi F, Muckenthaler MU, Da Silva MC, Balla G, Balla J, Jeney V. Atherogenesis and iron: from epidemiology to cellular level. Front Pharmacol. Switzerland; 2014;5:94.

45. Vinchi F, Costa da Silva M, Ingoglia G, Petrillo S, Brinkman N, Zuercher A, et al. Hemopexin therapy reverts heme-induced proinflammatory phenotypic switching of macrophages in a mouse model of sickle cell disease. Blood. United States; 2016 Jan;127(4):473–86.

46. Chai X, Zhao M, Li D, Meng J, Lu W, Mu J, et al. [Establishment of an mouse model of iron-overload and its impact on bone marrow hematopoiesis]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. China; 2013 Oct;35(5):547–52.

47. Zhang Y, Zhai W, Zhao M, Li D, Chai X, Cao X, et al. Effects of iron overload on the bone marrow microenvironment in mice. PLoS One. United States; 2015;10(3):e0120219.

48. Chai X, Li D, Cao X, Zhang Y, Mu J, Lu W, et al. ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice. Sci Rep. England; 2015 May;5:10181.

49. Tataranni T, Agriesti F, Mazzoccoli C, Ruggieri V, Scrima R, Laurenzana I, et al. The iron chelator deferasirox affects redox signalling in haematopoietic stem/progenitor cells. Br J Haematol. England; 2015 Jul;170(2):236–46.

50. Gattermann N, Finelli C, Porta M Della, Fenaux P, Stadler M, Guerci-Bresler A, et al. Hematologic responses to deferasirox therapy in transfusion-dependent patients with myelodysplastic syndromes. Haematologica. 2012;97(9):1364–71.

51. Angelucci E, Santini V, Di Tucci AA, Quaresmini G, Finelli C, Volpe A, et al. Deferasirox for transfusion-dependent patients with myelodysplastic syndromes: safety, efficacy, and beyond (GIMEMA MDS0306 Trial). Eur J Haematol [Internet]. 2014 Mar 3 [cited 2014 May 7];92(February 2014):527–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24580147

52. Visani G, Guiducci B, Giardini C, Loscocco F, Ricciardi T, Isidori A. Deferasirox improves hematopoiesis after allogeneic hematopoietic SCT. Bone Marrow Transpl [Internet]. Macmillan Publishers Limited; 2014 Apr;49(4):585–7. Available from: http://dx.doi.org/10.1038/bmt.2013.213

53. Pilo F, Angelucci E. A storm in the niche: Iron, oxidative stress and haemopoiesis. Blood Rev. England; 2017 Aug;

54. Angelucci E, Cianciulli P, Finelli C, Mecucci C, Voso MT, Tura S. Unraveling the mechanisms behind iron overload and ineffective hematopoiesis in myelodysplastic syndromes. Leuk Res. England; 2017 Nov;62:108–15.

55. Remacha ÁF, Arrizabalaga B, Villegas A, Durán MS, Hermosín L, de Paz R, et al. Evolution of iron overload in patients with low-risk myelodysplastic syndrome: iron chelation therapy and organ complications. Ann Hematol. 2015;94(5):779–87.

56. Gattermann N, Finelli C, Porta MD, Fenaux P, Ganser A, Guerci-Bresler A, et al. Deferasirox in iron-overloaded patients with transfusion-dependent myelodysplastic syndromes: Results from the large 1-year EPIC study. Leuk Res [Internet]. 2010;34(9):1143–50. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20451251

57. Nolte F, Höchsmann B, Giagounidis A, Lübbert M, Platzbecker U, Haase D, et al. Results from a 1-year, open-label, single arm, multi-center trial evaluating the efficacy and safety of oral Deferasirox in patients diagnosed with low and int-1 risk myelodysplastic syndrome (MDS) and transfusion-dependent iron overload. Ann Hematol [Internet]. 2013;92(2):191–8.

Available from: http://dx.doi.org/10.1007/s00277-012-1594-z

58. de Swart L, Hendriks JCM, van der Vorm LN, Cabantchik ZI, Evans PJ, Hod EA, et al. Second international round robin for the quantification of serum non-transferrin-bound iron and labile plasma iron in patients with iron-overload disorders. Haematologica. 2015;101(1):38–45.

59. Mainous AG, Tanner RJ, Hulihan MM, Amaya M, Coates TD. The impact of chelation therapy on survival in transfusional iron overload: a meta-analysis of myelodysplastic syndrome. Br J Haematol [Internet]. 2014;167(5):720–3. Available from: http://dx.doi.org/10.1111/bjh.13053

60. Leitch HA, Parmar A, Wells RA, Chodirker L, Zhu N, Nevill TJ, et al. Overall survival in lower IPSS risk MDS by receipt of iron chelation therapy, adjusting for patient-related factors and measuring from time of first red blood cell transfusion dependence: an MDS-CAN analysis. Br J Haematol. England; 2017 Oct;179(1):83–97.

61. Angelucci E, Anna S, Urru M, Pilo F, Piperno A. Myelodysplastic Syndromes and Iron Chelation Therapy. 2017;

62. List AF, Baer MR, Steensma DP, Raza A, Esposito J, Martinez-Lopez N, et al. Deferasirox reduces serum ferritin and labile plasma iron in RBC transfusion-dependent patients with myelodysplastic syndrome. J Clin Oncol. 2012;30(17):2134–9.

63. Daar S, Pathare A, Nick H, Kriemler-Krahn U, Hmissi A, Habr D, et al. Reduction in labile plasma iron during treatment with deferasirox, a once-daily oral iron chelator, in heavily iron-overloaded patients with beta-thalassaemia. Eur J Haematol. England; 2009 Jun;82(6):454–7.

64. Cermak J, Jonasova A, Vondrakova J, Cervinek L, Belohlavkova P, Neuwirtova R. A comparative study of deferasirox and deferiprone in the treatment of iron overload in patients with myelodysplastic syndromes. Leuk Res. England; 2013 Dec;37(12):1612–5.

65. Nick H, Acklin P, Lattmann R, Buehlmayer P, Hauffe S, Schupp J, et al. Development of tridentate iron chelators: from desferrithiocin to ICL670. Curr Med Chem. Netherlands; 2003 Jun;10(12):1065–76.

66. Cappellini MD, Cohen A, Piga A, Bejaoui M, Perrotta S, Agaoglu L, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood. United States; 2006 May;107(9):3455–62.

67. Vichinsky E, Onyekwere O, Porter J, Swerdlow P, Eckman J, Lane P, et al. A randomised comparison of deferasirox versus deferoxamine for the treatment of transfusional iron overload in sickle cell disease. Br J Haematol. England; 2007 Feb;136(3):501–8.

68. Kohgo Y, Urabe A, Kilinc Y, Agaoglu L, Warzocha K, Miyamura K, et al. Deferasirox Decreases Liver Iron Concentration in Iron-Overloaded Patients with Myelodysplastic Syndromes, Aplastic Anemia and Other Rare Anemias. Acta Haematol. Switzerland; 2015;134(4):233–42.

69. Shah NR. Advances in iron chelation therapy: transitioning to a new oral formulation. Drugs Context. England; 2017;6:212502.

70. Nolte F, Angelucci E, Breccia M, Gattermann N, Santini V, Vey N, et al. Updated recommendations on the management of gastrointestinal disturbances during iron chelation therapy with Deferasirox in transfusion dependent patients with myelodysplastic syndrome - Emphasis on optimized dosing schedules and new formulations. Leuk Res. England; 2015 Oct;39(10):1028–33.

71. Taher AT, Origa R, Perrotta S, Kourakli A, Ruffo GB, Kattamis A, et al. New film-coated tablet formulation of deferasirox is well tolerated in patients with thalassemia or lower-risk

MDS: Results of the randomized, phase II ECLIPSE study. Am J Hematol. United States; 2017 May;92(5):420–8.

72. Porter J, Galanello R, Saglio G, Neufeld EJ, Vichinsky E, Cappellini MD, et al. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): A 1-yr prospective study. Eur J Haematol. 2008;80(2):168–76.

73. Gattermann N, Jarisch A, Schlag R, Blumenstengel K, Goebeler M, Groschek M, et al. Deferasirox treatment of iron-overloaded chelation-naïve and prechelated patients with myelodysplastic syndromes in medical practice: Results from the observational studies eXtend and eXjange. Eur J Haematol. 2012;88(3):260–8.

74. Gale RP, Barosi G, Barbui T, Cervantes F, Dohner K, Dupriez B, et al. What are RBC-transfusion-dependence and -independence? Leukemia research. England; 2011. p. 8–11.

75. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood. 2005.

28. APPENDICES

28.1. APPENDIX A: ECOG Performance Status

SOURCE: *Oken MM et al, Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.*

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

28.2. APPENDIX B: Common Terminology Criteria for Adverse Events (CTCAE)

In the present study, adverse events and/or adverse drug reactions will be recorded according to:

Common Terminology Criteria for Adverse Events (CTCA), version 5.0.

At the time this protocol was issued, the full CTC document was available on the NCI web site, at the following address:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

28.3. APPENDIX C: Questionnaire-QOL

http://qol-e.com/MDS.asp

28.4. APPENDIX D: Questionnaire- CIRS

Parmelee PA, Thuras PD, Katz IR, Lawton MP. Validation of the Cumulative Illness Rating Scale in a geriatric residential population J Am Geriatr Soc. 1995 Feb;43(2):130-7.

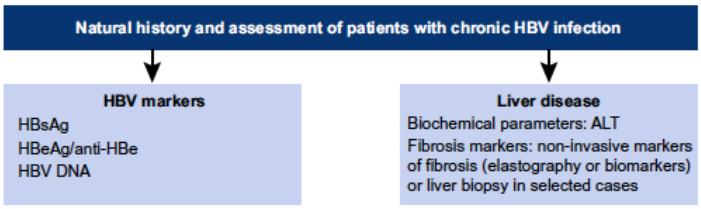
28.5. APPENDIX E: IPSS and Revised IPSS

IPSS: (https://qxmd.com/calculate/calculator_123/mds-intnl-prognostic-scoring-sys-ipss)
Revised IPSS: https://www.mds-foundation.org/ipss-r-calculator/

28.6. APPENDIX F: EASL criteria for Chronic hepatitis EASL 2017 Clinical Practice

Guidelines on the management of hepatitis B virus infection. European Association for the Study of the Liver.
Electronic address: easloffice@easloffice.eu
J Hepatol. **2017** Aug;67(2):370-398. doi: 10.1016/j.jhep.**2017**.03.021. Epub **2017** Apr 18. PMID: 28427875

JOURNAL OF HEPATOLOGY



	HBeAg positive		HBeAg negative	
	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis
HBsAg	High	High/intermediate	Low	Intermediate
HBeAg	Positive	Positive	Negative	Negative
HBV DNA	>10^7 IU/ml	10^4-10^7 IU/ml	<2,000 IU/ml°°	>2,000 IU/ml
ALT	Normal	Elevated	Normal	Elevated*
Liver disease	None/minimal	Moderate/severe	None	Moderate/severe
Old terminology	Immune tolerant	Immune reactive HBeAg positive	Inactive carrier	HBeAg negative chronic hepatitis

Fig. 1. Natural history and assessment of patients with chronic HBV infection based upon HBV and liver disease markers. *Persistently or intermittently. °°HBV DNA levels can be between 2,000 and 20,000 IU/ml in some patients without sings of chronic hepatitis.

28.7. APPENDIX G: Transferrin saturation calculation

[Serum iron / (serum transferrina x 1.42)] x 100

Serum iron (microgr/dl), Serum transferrin (mg/dl)]

www.emocromatosi.it/test.asp

28.8. APPENDIX H: Sampling, conservation and shipment modality for centralized tests

Sampling for NTBI and LPI and other centralized tests

Baseline tests sampling must be performed before any chelation therapy and as far as possible before PRBC transfusion (ideally before transfusion). All test during the year of therapy must be obtained as far as possible the daily DFX assumption (before daily DFX assumption) and as far as possible any transfusional event (distance from transfusion must be recorded). Distance from DFX assumption and transfusional event must be registered.

A certified central laboratory will be utilized to process and provide results for the experimental laboratory tests (NTBI/LPI/MDA/ epcidine/ heritroferrone/ GDF11 , GDF 15/ sTR ecc).(for evaluation tests list and collection time-point see TAB 15.7)

Every laboratory chosen for this study will provide instruction regarding the collection, processing and shipment of appropriate simples.

Others samples will be referred to the local lab. (for evaluation tests list and collection time-point see TAB 15.6)

- Hematology CBC sample collection 4mL blood
- Biochemistry sample collection 8,5 mL blood
- Serum ferritin 8,5 mL blood
- Urine analysis sample collection 10 mL urine

All samples should be obtained in the absence of known inflammation or infection. If transfusion is scheduled, blood should be drawn for serum collection prior to transfusion. No indication regarding daily drug administration (other DFX) and samples collections.

Conservation

Every laboratory chosen for this study will provide instruction regarding the collection, processing, preservation and shipment of appropriate simples.

Shipment

Every laboratory chosen for this study will provide instruction regarding the collection, processing, preservation and shipment of appropriate samples.

28.9. APPENDIX I: Image acquisition guidelines for liver/spleen/pancreas MRI

- **The table below shows <u>preferred</u> parameters. Use parameters as close to these as possible.**
- **Once these values have been selected, they must remain consistent throughout this examination and study follow-up examinations.**
- **The Same Imaging Protocol Must Be Used at all Visits**

Scanner Type	**1.5 T (GE, Siemens, Philips)** **Important Reminder:** Please save an optimized MRI protocol for the study on the scanner and use consistent parameters across all time points.	
Anatomical coverage (Required Anatomy)	**Localizer must be used to get liver, spleen and pancreas – this will serve as the reference scan. T2*w protocol must be slice matched with this anatomical scan. Acquire 3 slices to capture liver, spleen and pancreas. Please make sure each organ is captured within at least one slice.**	
Sequence details	**Localizer**	**T2*w BREATHHOLD**
Contrast Agent	NO contrast agent shall be administered during/before/after any scan for this MRI protocol	
Pulse sequence	Fast Spin Echo	Multi-Echo FGRE/FFE with <u>fat suppression</u>
RF coil	Phased-Array Torso Coil	
Plane	Axial	
TR (ms)	1000 – 4000 (or optimal)	200 (or optimal)
TE (ms)	65 (or optimal)	**Preferred:** Multi-Echo FGRE/FFE with <u>fat suppression</u> **Min TE possible (maximum value can be 1ms) , 8 to 12 echoes, 2ms increments** **For example: TEs = 1, 3, 5, 7, 9, 11, 13, 15**
Flip angle (degrees)	Optimal	20
Slice thickness (mm)	15	15
Slice gap (mm)	5	5
Matrix (Frq x Ph)	320 x 192 (or optimal)	96 X 128
FOV (cm)	32 – 44 (or optimal to include all anatomy)	32-44 (or optimal to include desired anatomy)
Fat suppression	-	**ON**
Receiver bandwidth	Optimal	Optimal