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MASSIVE IRON DEPOSIT ASSESSMENT

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MIDAS: MASSIVE IRON DEPOSIT ASSESSMENT

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Brief Overview

Iron overload is a severe complication of multiple blood transfusions. As the body has no physiologic mechanism for clearing iron, repeated transfusions cause iron accumulation in organs and lead to iron toxicity. Accurate assessment of iron overload is paramount to quantify excessive iron accumulation and to monitor response to iron chelation therapy. Magnetic resonance imaging (MRI) methods have been used to noninvasively measure hepatic iron concentration (HIC). Although MRI-based measurements of transverse relaxation rates (R2 and R2*) accurately predict biopsy-proven HICs below 15 mg Fe/g, previous studies have shown that their precision is limited for HICs above 15 mg Fe/g and inaccurate above 25 mg Fe/g. Current R2* gradient-echo (GRE) MR techniques fail occasionally for very high iron overloads (HIC ~ 15-25 mg Fe/g) and always for massive iron overloads (HIC > 25 mg Fe/g) because R2* is so high that the MR signal decays before it can be measured accurately.

The Massive Iron Deposit Assessment (MIDAS) study aims to extend the clinically useful range of R2*-based HIC measurements by employing ultra short echo time (UTE) imaging to improve sampling of the relaxation curve. UTE shortens the earliest sampling time, or echo time (TE), of the R2*-measurement sequence, allowing detection of very fast signal decay and a higher R2* fit precision. The MIDAS study will test the accuracy of R2*-UTE in estimating HIC in massively iron-overloaded research participants. R2*-UTE sequence will be tested over a wide range of R2* values and in healthy volunteers. Research participants will be scanned with R2*-GRE and R2*-UTE MRI. Massively iron-overloaded research participants are expected to fail the R2*-GRE screen and may require a clinically indicated liver biopsy for iron quantification. Study data of this cohort will be used for modeling R2*-UTE using HIC by liver biopsy as the reference method.

The biopsy-calibrated R2*-UTE technique will provide a noninvasive, accurate, and cost-effective alternative to biopsy for HIC quantification in massively iron-overloaded research participants and ensure appropriate dosing of intensive iron unloading treatment to this group. This will reduce treatment-related toxicity arising from unnecessary exposure to iron chelation and significantly improve research participants' care and quality of life.

Objective:

To test the association of hepatic iron content (HIC) measured with the newly developed 1.5T R2*-UTE technique and HIC quantified by liver biopsy in subjects with iron overload.

Responsible Investigators: Jane Hankins, MD, MS and Claudia Hillenbrand, PhD

MIDAS: MASSIVE IRON DEPOSIT ASSESSMENT

Estimated date for completion of data collection: 4 years after start of study.
Estimated start date April 2012, objective will be completed April 2016.

Hypotheses/Estimates: The biopsy-calibrated R2*-UTE technique will provide a noninvasive, accurate, and cost-effective alternative to biopsy for HIC quantification in massively iron-overloaded research participants**.**

Criteria for Evaluation: To compare the results of the new UTE MRI with that of a liver biopsy in research participants with extremely high amounts of iron in the liver

Study Design: This will be a non-therapeutic, prospective study aimed to calibrate a novel R2*-UTE imaging sequence technique to non-invasively and accurately estimate high hepatic iron content (HIC) values

Study Population:

Inclusion Criteria:

Patients:

1. History of 12 or more lifetime erythrocyte transfusions, and
2. Need for liver iron content assessment

Healthy volunteers:

1. Willingness to undergo MRI testing for investigation and implementation of the R2*-UTE technique
2. 18 years of age or older

Exclusion Criteria:

Patients:

1. Presence of certain MR-unsafe foreign material in the body, or other conditions that make the patient ineligible for an MRI scan per St. Jude policies.
2. Any condition or chronic illness that in the opinion of the PIs makes participation on study ill-advised.

Healthy volunteers:

- History of blood transfusions in the lifetime
- Known history of hereditary hemochromatosis
- Presence of certain foreign, MR-unsafe material in the body, or other conditions that make the volunteer ineligible for an MRI scan per St. Jude policies.
- Any condition or chronic illness that in the opinion of the PIs makes participation on study ill-advised.

Sample Size: Approximately 200 research participants

Data Analyses:

Planned Analyses for Primary Study Aim:

To explore the association of hepatic iron content (HIC) measured with the newly developed 1.5T R2*-UTE technique and HIC quantified by liver biopsy in subjects with iron overload.

The relationship of HIC measured with 1.5T R2*-UTE and quantified by liver biopsy in subjects with iron overload will be explored graphically. If the linear relationship exists, the

Pearson's correlation between the HIC measured with 1.5T R2*-UTE technique and the HIC quantified by liver biopsy will be calculated and its 95% confidence interval (CI) will also be reported. If the correlation is sufficiently high, i.e., the lower bound of the 95% CI exceeds 0.7, a simple linear regression model will be established for the HIC prediction. The 95% prediction bounds will be established. If more complicated pattern shown graphically, appropriate model will be developed to describe the relationship.

Anticipated Study Completion Date: April 2017

Timeframe for Primary Outcome Measure: There will be 2 defined intervals for this study and 1 possible: (I) 1.5 and 3T MRI's, and (II) liver biopsy.

Defined Interval:

1. Screening test with the regular R2*-GRE MRI
    - The new UTE MRI will be done at the same time as the regular R2*-GRE MRI

   OR
2. Liver biopsy

Possible Interval (if liver biopsy not done first, within 30 days):

1. If the regular R2*-GRE MRI can measure the amount of iron in the liver, no other tests will be done.
2. If the regular GRE MRI cannot measure the amount of iron in the liver because the amount of iron is extremely high, a liver biopsy will be recommended to measure the exact amount of iron in the liver.

The primary objective of this study is to compare the results of the new UTE MRI with that of a liver biopsy in research participants with extremely high amounts of iron in the liver.

Data Management: Data collection and data management for this investigation will be conducted through the SJCRH Hematology Department. Co-investigators and CRAs assigned to this protocol will be responsible for assisting the PI in assuring protocol compliance as well as reviewing, transcribing and tracking of all clinical and safety related data.

Human Subjects: MRI does not involve radiation, therefore there are no risks associated with radiation injury. Study participants may need to be sedated for the MRI exam, which may expose the patients to the risks involved with anesthetics and airway complication.

R2*MRI is done on a regular basis and the additional R2*UTE exams that will be offered in MIDAS will be an additional 30 minutes of MRI table time at 1.5T. Subjects may experience anxiety and claustrophobia during MRI exams, however. If this occurs, the study will be temporarily interrupted, and the subject will be given instructions on how to best tolerate the exam and offered to watch movie or listen to music to help with the relaxation process during the test. In addition, St. Jude Child Life Services have worked very closely with our pediatric subjects and have helped by offering distraction techniques which have improved the tolerance to not only MRI tests, but many other imaging studies. If even after all these measures the study subject is still not able to tolerate the MRI study, the study will

be interrupted.

Abstract

Iron overload is a severe complication of multiple blood transfusions. As the body has no physiologic mechanism for clearing iron, repeated transfusions cause iron accumulation in organs and lead to iron toxicity. Accurate assessment of iron overload is paramount to quantify excessive iron accumulation and to monitor response to iron chelation therapy. Magnetic resonance imaging (MRI) methods have been used to noninvasively measure hepatic iron concentration (HIC). Although MRI-based measurements of transverse relaxation rates (R2 and R2*) accurately predict biopsy-proven HICs below 15 mg Fe/g, previous studies have shown that their precision is limited for HICs above 15 mg Fe/g and inaccurate above 25 mg Fe/g. Current R2* gradient-echo (GRE) MR techniques fail occasionally for very high iron overloads (HIC ~ 15-25 mg Fe/g) and always for massive iron overloads (HIC > 25 mg Fe/g) because R2* is so high that the MR signal decays before it can be measured accurately.

The Massive Iron Deposit Assessment (MIDAS) study aims to extend the clinically useful range of R2*-based HIC measurements by employing ultra short echo time (UTE) imaging to improve sampling of the relaxation curve. UTE shortens the earliest sampling time, or echo time (TE), of the R2*-measurement sequence, allowing detection of very fast signal decay and a higher R2* fit precision. The MIDAS study will test the accuracy of R2*-UTE in estimating HIC in massively iron-overloaded research participants. R2*-UTE sequence will be tested in phantoms over a wide range of R2* values and in healthy volunteers. Research participants will be scanned with R2*-GRE and R2*-UTE MRI. Massively iron-overloaded research participants are expected to fail the R2*-GRE screen and may require a clinically indicated liver biopsy for iron quantification. Study data of this cohort will be used for modeling R2*-UTE using HIC by liver biopsy as the reference method.

The biopsy-calibrated R2*-UTE technique will provide a noninvasive, accurate, and cost-effective alternative to biopsy for HIC quantification in massively iron-overloaded research participants and ensure appropriate dosing of intensive iron unloading treatment to this group. This will reduce treatment-related toxicity arising from unnecessary exposure to iron chelation and significantly improve research participants' care and quality of life.

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1.0 OBJECTIVES

1.1 Primary Objective

To test the association of hepatic iron content (HIC) measured with the newly developed 1.5T R2*-UTE technique and HIC quantified by liver biopsy in subjects with iron overload.

1.2 Secondary Objectives

1.2.1 To explore the relationship between 1.5T R2*-UTE and 1.5T R2*-GRE measurements in healthy volunteers and in subjects with iron overload.

1.2.2 To explore the relationship between 1.5T R2*-UTE measurements with iron studies (serum iron and transferrin saturation) in subjects with iron overload.

1.3 Exploratory Objectives

1.3.1. To explore the relationship between hepatic 3T R2*-UTE measurements and HIC obtained by liver biopsy in subjects with massive iron overload

1.3.2. To explore the relationship between 1.5T R2*-UTE and 3T R2*-GRE measurements in healthy volunteers and in subjects with iron overload

1.3.3. To explore the relationship between 3T R2*-UTE and 3T R2*-GRE measurement in healthy volunteers and in subjects with iron overload

1.3.4. To explore the relationship between 1.5T R2*-UTE and 3T R2*-UTE measurements in healthy volunteers and in subjects with iron overload

1.3.5. To explore the relationship between 3T R2*-UTE measurements with iron studies (serum iron and transferring saturation) in subjects with iron overload

2.0 BACKGROUND AND RATIONALE

2.1 Iron Overload

Iron overload or excessive body iron burden is a serious condition resulting from increased dietary gastrointestinal absorption, multiple erythrocyte transfusions, or both. Increased intestinal iron uptake leading to significant iron deposits is seen in hereditary hemochromatosis and also in hematologic conditions with ineffective erythropoiesis. Repeated erythrocyte transfusions are used in many hematologic diseases to either supply red blood cells when there is ineffective bone marrow erythropoiesis [e.g., thalassemia major (TM), myelodysplastic syndromes, Diamond-Blackfan anemia (DBA), congenital dyserythropoietic anemia] or to prevent complications of the disease [e.g., sickle cell disease (SCD)], by suppressing the bone marrow's production of red blood cells or supplying functionally normal ones.[1;2] Each milliliter of transfused blood has

approximately 1 mg of iron. In the balanced state, only 1–2 mg of iron enters and leaves the body each day. With repeated transfusions, iron accumulates at a very fast rate as there is no physiologic mechanism for its elimination. Patients undergoing chronic transfusions have an iron excess of 0.3–0.4 mg/kg/day, and laboratory signs of iron overload can be detected after 15–20 repeated transfusions.[3]

Excess iron accumulates in nearly all tissues, but most notably in the liver, heart, thyroid, kidneys, spleen, pituitary gland, and pancreas. The normal HIC value in the liver is less than 2.4 mg Fe/g dry weight. Liver is one of the main target organs in iron overload. During the process of iron loading, iron deposition in the liver is substantial; therefore, hepatic disease is a very common finding of systemic iron overload. The degree of liver dysfunction is directly dependent on the amount of hepatic iron deposition. Progressive iron accumulation eventually leads to hepatomegaly, liver synthetic abnormalities, fibrosis, and finally, cirrhosis and liver failure.[4-8] Also, the risk of developing hepatocellular carcinoma increases significantly in patients with cirrhosis secondary to iron overload.[9] Cardiac failure is a complication of major concern in patients with increased body iron burden. Myocardial iron deposition leads to arrhythmias and progressive heart dysfunction, and remains the most important cause of death in patients with TM.[10] Myocardial hemosiderosis can also occur in other hematologic diseases treated with repeated blood transfusions, such as DBA, and less frequently in SCD. Cardiac dysfunction is associated with the degree of iron deposition in the myocardium,[11] and HIC values persistently ≥ 15 mg Fe/g of liver dry weight are associated with increased cardiac morbidity and early death in patients with TM.[12] Hepatic iron can accumulate to extreme amounts, with HIC values more than 67 mg Fe/g being reported, placing these massively iron-overloaded patients in great danger of the toxicities of iron.[13-16]

In summary, progressive iron accumulation can lead to increased deposition in tissues of key organs such as the heart, liver, and other tissues, causing organ dysfunction, substantial morbidity, and increased mortality. Progressive iron accumulation can raise HIC to dangerous levels, placing patients at very high risk for the toxic effects of excessive iron.

2.2 Assessment of Iron Overload

Liver Biopsy. Accurate assessment of the body iron burden is essential to manage iron-overloaded patients properly by instituting measures to unload iron, such as initiating iron chelation therapy or therapeutic phlebotomy (in case of non-transfusion–dependent patients), or adjusting the dose and intensity of chelation. In addition, close monitoring of the body iron burden can help avoid the adverse effects of excessive iron chelation. Annual or more frequent assessments of iron burden are necessary for appropriately monitoring of body iron burden. Because HIC consistently mirrors total body iron, the chemical analysis of hepatic specimens obtained through needle biopsy is considered the reference method to evaluate iron excess in systemic iron overload.[17] Liver biopsy, however, is an invasive procedure that carries risks such as pain, bleeding, and infection, in turn leading to suboptimal patient adherence.

Serum Ferritin. Serum ferritin is used as an indirect measure of iron overload, but can overestimate the body iron stores because it increases in autoimmune, infectious, and inflammatory conditions.[18-21] Serum ferritin correlates poorly with HIC, and important therapeutic decisions should not be based solely on one measure.[14;22] Serial serum ferritin

measurements can provide a general idea of the degree of iron overload; however, serum ferritin trends can also incorrectly estimate iron storage. There is evidence that serum ferritin trends can under- or overestimate the total iron body content, significantly and dangerously compromising adequate medical management of iron overload.[6;23-26]

Imaging Methods. Accurate and noninvasive assessment of body iron burden is essential for proper medical management of patients with iron overload. Currently, two noninvasive methods in clinical use have been calibrated with liver biopsy: (1) measurement of biomagnetic susceptometry by using a superconducting quantum interference device (SQUID),[27] and (2) determination of liver tissue relaxivity rates (R2 and R2*) by MRI.[11;16;28-30] SQUID has a reduced accessibility as there are only 4 systems installed worldwide. Hence, liver tissue MRI relaxivity is currently the most commonly studied technique to diagnose and monitor iron overload noninvasively. Although MRI relaxivity techniques can accurately predict HIC, their predictive power progressively decreases as HIC values increase.[11;16;29;31]

2.3 Massive Iron Overload and Absence of an Accurate Noninvasive Quantitation Method

Massive iron hepatic deposits are a common disorder in patients with iron overload. Published data and findings from an a recent multicenter trial for iron-overloaded patients (SWiTCH ,www.clinicaltrials.gov # NCT00122980), show a high prevalence of patients with very elevated HIC values, with approximately 40% of patients having biopsy-confirmed HIC ≥15 mg Fe/g (Table 1).[16;29;32;33]

Table 1: Distribution of HIC values in patients with iron overload.

Study	*N*	*HIC (mg Fe/g)*			*Highest HIC (mg Fe/g)*
		≥15	≥20	≥25	
Ferritin and liver biopsy comparison study[13]	39		10 (26%)		67.97
SWiTCH study[34]	145	64 (44%)	47 (32%)	24 (17%)	61.3
Calibration of MRI R2 and R2*[15]	22			7 (32%)	57.8
Thalassemia Clinical Research Network[34]	166	55 (33%)			43
Calibration of MRI R2[29]	105			12 (12%)	42.7
Calibration of MRI[33]	26			6 (23%)	not provided
STOP study[23]	39	26 (39%)		10 (26%)	41.3

Although different groups have successfully calibrated R2 and R2*-MRI techniques with liver biopsy-proven HIC,[11;16;29] no previously developed technique has been able to accurately quantify HIC in patients with massive iron overload because of the exceedingly fast signal decay produced by high concentrations of tissue iron, which cannot be captured with current R2-spin

echo (SE) and R2*-gradient echo (GRE) techniques. The prediction accuracy that can be achieved with an R2*-GRE iron quantification sequence – which is favored over R2-SE because of the short acquisition time of a single breath-hold – deteriorates when signal decay (1/R2*) is about the echo time (TE) of the first echo and the signal of the first echo falls near the noise level.[16] In other words, the error in predicting iron values for R2* > 500 Hz (~15 mg Fe/g) is significant, but even higher for R2* > 900 Hz (~25 mg Fe/g).[33] Also, the minimum TE of conventional MR sequences cannot be much shorter than 1 ms for technical reasons. The only method to obtain accurate estimates of iron concentration in patients with extremely high R2* values (R2* > 500 Hz, HIC > 15 mg Fe/g) in a typical measurement time of a single or a few breath-holds is to shorten the minimum TE substantially, which has not been previously tried for quantifying tissue iron.

2.4 Risk of Inappropriate Monitoring of Iron-Chelation Therapy in Heavily Iron-Overloaded Patients

In patients undergoing chronic transfusion therapy, HIC values should be maintained between 5 and 7 mg Fe/g in order to prevent the toxic effects of tissue iron deposition.[3] Iron chelators and therapeutic phlebotomy can provide absolute HIC reductions of approximately 2–5 mg Fe/g per year.[13;35] Because current R2*-GRE techniques lose accuracy beyond HIC values of 15 mg Fe/g and are insensitive to quantify HIC values of approximately 25 mg Fe/g or higher, chelation monitoring is difficult in patients with massive HIC values. For example, a patient with an HIC of 60 mg Fe/g can maximally unload iron at 5 mg Fe/g per year; therefore, with the current MRI technology, it would take 7 years to obtain a trustable R2*-based HIC measurement. This long delay in quantifying HIC is not clinically acceptable and prevents optimal clinical management of massively iron-overloaded patients for inappropriately long times. Current MRI technology cannot correctly quantify high HIC levels in massively iron-overloaded patients, who are at increased risk for the toxic effects of tissue iron accumulation. Massive iron deposits typically require a more aggressive management using iron chelation therapy, which is often difficult because of inaccurate iron quantification. Different strategies have been used for highly iron-overloaded patients, such as high doses of iron chelators (e.g., up to 40 mg/kg/day of the novel oral iron chelator deferasirox),[36] intravenous continuous infusion of deferoxamine, or combinations of different iron chelators.[36-39] However, such aggressive approaches can cause increased drug-related toxicity, such as renal, hepatic, ophthalmologic, and auditory damage, making close monitoring with accurate quantitation of tissue iron critical. In the absence of an adequate measure to quantify tissue iron, massively iron overloaded patients may receive iron unloading therapy at inappropriately high doses or for unnecessarily long periods of time, thereby placing them at risk to toxic effects of this therapy.

2.5 Ultra short Echo Time R2* for Accurate Assessment of Massive Iron Deposits

To obtain accurate estimates of iron concentration in patients with extremely high R2* values, the minimum TE needs to be less than 1 ms. This can be accomplished by using a different scanning approach, such as the ultra short echo time (UTE) technique.[40;41] UTE pulse sequences have TEs that are on average 25 times shorter than the shortest TEs achieved by conventional clinical R2* imaging sequences.[40;42] Their use therefore allows visualization of tissue with extremely high relaxivity rates, which cannot otherwise be detected by MRI. UTE was originally proposed for acquiring lung images[40] and then applied to the tendon,[41;43;44]cortical bone,[41;45-

[49]spine,[50;51] and liver.[52] Exploratory work on R2* mapping has been reported, e.g. for ice ball imaging during cryoablation,[53] cartilage imaging,[54] or ex-vivo imaging of arteriosclerotic lesions.[55] The limiting factor for TE in conventional Cartesian imaging is the time needed for phase encoding and for refocusing the slice selection gradient. UTE sequences use radial sampling (back projection imaging) or spiral trajectories,[54] to bypass phase-encoding gradients.[56;57] Slice-selective (2D) UTE sequences employ half radio frequency (RF) pulses, which do not need a slice refocusing pulse.[58]

Since SQUID is not broadly available and because the existing R2*-GRE technique cannot accurately quantify very high concentrations of iron in tissues, MRI techniques using UTE sequences are a clinically important alternative for accurate iron quantification in cases of massive iron overload. R2*-UTE – which has not been previously implemented for tissue iron quantitation – can be suitable to quantify hepatic iron at not only normal and moderately elevated ranges of HIC values, but also at very high physiologic ranges (> 25 mg Fe/g).

2.6 Calibration of the R2*-GRE Technique – the MRIRON Study

Since 2005, we have investigated MR quantification of patients with transfusional iron overload at St. Jude Children's Research Hospital. As part of the institutional MRIRON trial,[33] we studied 43 research participants with iron overload [sickle cell anemia (32), β-thalassemia major (6), and bone marrow failure (5)] who completed liver biopsy within 30 days of the R2*-GRE examination. Their median age was 14 years, median transfusion duration 15 months, average (±SD) serum ferritin 2718 ± 1994 ng/mL, and average HIC 10.9 ± 6.8 mg Fe/g dry weight liver. Our R2*-GRE sequence acquired 20 axial liver images with increasing echo times (TE=1.1–17.3 ms) in a single breath-hold. The signal intensity decay over the 20 images in each pixel was measured as a function of TE and quantitative T2* (T2*=1/R2*) maps were calculated with an exponential fit after background subtraction. Three independent reviewers performed ROI analysis on T2* maps, and their results were in excellent agreement (intraclass correlation coefficient = 0.98). We found serum ferritin and R2*-GRE to be weakly but significantly associated (correlation coefficients 0.41–0.48, all $P < .01$) and R2*-GRE to be strongly associated with HIC (correlation coefficients 0.96–0.98, all $P < .0001$). This high correlation confirmed results of prior reports,[11, 15] calibrated R2*-GRE measurements, and demonstrated the clinical feasibility of predicting HIC using R2*-MRI. Figure 1 summarizes the measured data and HIC prediction curve.

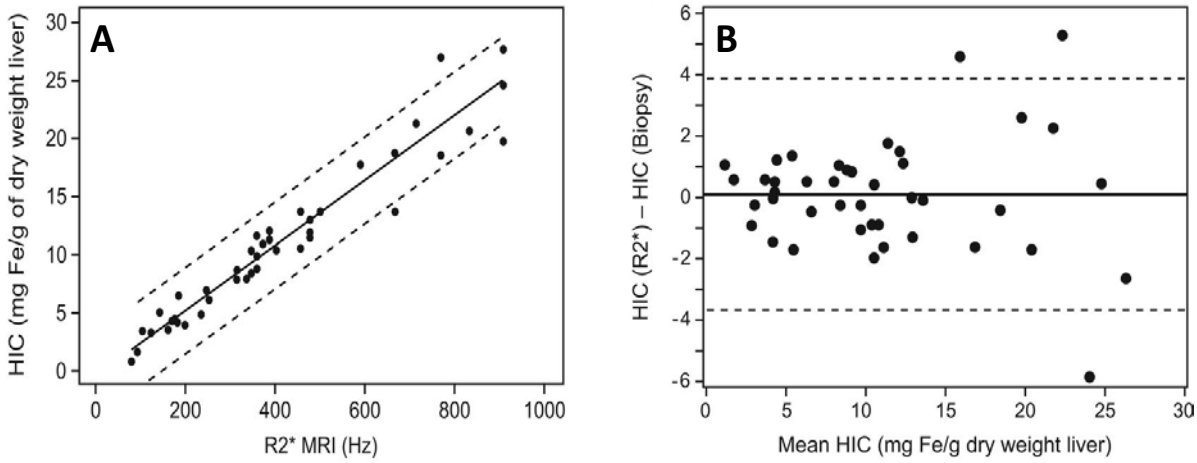

Figure 1: MRIRON study results: R2*-GRE vs. HIC. (A) Correlation between measured R2* values and HIC biopsy results with linear regression line (solid line) and 95% prediction limits (dashed lines). (B) Bland-Altman plot indicating the mean difference between HIC values predicted by R2*-GRE and biopsy (solid line) and the upper and lower 95% limits of agreement between the two measurements (dashed lines). The uncertainty of the prediction for higher HIC (>15mg Fe/g dry weight) is discussed in the text.[33]

On the basis of these results, R2*-GRE is now the standard of care for iron quantification of research participants at St. Jude. Since the introduction of R2*-GRE as clinical order at St. Jude in 2008, we have performed over 150 iron tests. Biopsies are performed at St. Jude only for research participants enrolled on a clinical protocol that requires liver biopsy or for those with clinical indication for liver biopsy (e.g., patients with massive iron overload, or for those whose architectural structure of the liver is needed).

Although the calibrated R2*-GRE method was successfully applied in the MRIRON study, it had shortcomings:

1. The T2* fit failed for massively overloaded research participants (liver iron content > 25 mg Fe/g) because the liver MR signal vanished before the first image of the R2*-GRE image series was acquired. The chi-square goodness-of-fit test rejected pixel values if the difference between measured and model data was more than 1% and/or the fit value for R2* was greater than 1000 Hz, either of which usually occurs for HIC values of 25 mg Fe/g or higher. Other investigators have also reported a breakdown of their fitting routines at approximately 700–1000 Hz.[11;59] Therefore, current R2*-MRI methods can only detect *massive* iron deposit in the liver, but not quantify it accurately. Without biopsies, the clinician has to manage chelation therapy blindly until there is unloading into the "MR sensitive" range of HIC.
2. MRIRON research participants with HIC values between 15 and 25 mg Fe/g (R2* = 500–900 Hz) displayed larger deviations from the prediction curve (up to ± 6 mg Fe/g; ~30%; see Fig. 1B), which limits the accuracy of the method and can be potentially risky to patients if these numbers under- or overestimate true HIC value.

The precision of MR-based iron quantification and the range over which R2* can be precisely detected need to be increased for adequate clinical monitoring of highly iron-overloaded patients. Our preliminary studies indicate that increasing errors in R2*-GRE–based HIC quantification starting with an HIC of approximately 15 mg Fe/g (R2* > 500 Hz) are driven by fundamental limits on the precision of R2* estimated by curve fitting in pixels with high R2* relaxivity (high iron content).

2.7 Precision and Range of R2*-MRI–based HIC Quantification

The optimization of R2* measurements depends on several factors, such as the range of T2* times of interest, the MR technique used to estimate the T2* relaxation time, and the acquisition time available for the MRI scan.

Following the algorithms presented by Ogg and Kingsley[60] for optimized precision of T1 relaxation measurements, we derived a model that estimates the precision for T2* (or R2*) values calculated by iterative curve fitting with the Levenberg–Marquardt numerical algorithm for non–linear least square fitting.[61;62] For T2* decay, we assumed a 3-parameter model with

$$S_n = S_0 \cdot \exp(-TE_n/T2^*) + \text{noise},$$

where the signal S_n is the measured signal intensity of the n^{th} T2*-weighted image. The echo time TE_n of image $n$is the independent variable, and S_0 and T2* are model parameters. S_0 is determined by the experiment [e.g., proton density or flip angle – both primarily affecting the signal-to-noise ratio (SNR)], and T2* is the parameter of interest.

The precision of the T2* estimates, expressed as the coefficient of variation [CV = (standard deviation of T2*)/(mean of T2*)], was calculated from the Fisher information matrix of the model function.[60] Numerical simulations of the fit precision as function of R2* were performed using the MR parameters of the MRIRON R2*-GRE exam, which were chosen to be very similar to those of a previously published iron quantification study.[11] Specifically, our R2*-MRI model parameters are $n = 20$ images, $TE_1 = 1.1$ ms, $TE_n = TE_1 + (n - 1) \cdot 0.8$ ms. We used an SNR of 50 for the parameter S_0, the signal intensity at t=0 ms before T2* relaxation occurs. We derived this value from our MRIRON study image data by extrapolating the average liver SNR measurements to$t$=0 ms.

Figure 2 shows the 95% confidence interval for the T2* fit derived from the precision calculations and overlaid on the Bland–Altman plot in Fig. 1B. The simulation (red curve) shows that there is a nonlinear change in precision. This change is not easily recognized in the low to moderately iron-overloaded population (<15 mg/g), and therefore may have been missed by other investigators. The simulation confirmed our experimental observation of a large imprecision for higher iron overload with the standard R2*-GRE method. Other researchers have reported higher HIC values using standard R2*-GRE, but these data points demonstrated larger errors or deviated from the linear regression line.[16;59] Interpretations of these findings focus on the multi-exponential signal decay or nonlinear relationship between HIC and R2*-GRE, respectively, in highly overloaded areas of the liver.[59] Because the results of our fit precision simulations and experimental data are in concordance, we conclude that the fit imprecision at

higher HIC is the major source of inaccuracy between HIC and R2*-MRI while measuring iron overload in patients; other potential sources of error may be negligible.

Our simulation model gives the upper limit on the R2* fit precision obtained with a specific MR sequence (e.g., R2*-GRE). With the proposed R2*-UTE sequence, which yields optimized precision for high R2* values too, we can characterize other potential factors impacting the correlation between HIC and R2*. Although multi-exponential or nonlinear behavior cannot be ruled out by using current methods, a more accurate quantification of large R2* species can help determine the true correlation between HIC and R2*-MRI and ultimately the distribution of hepatic iron stores in patients with massive iron deposition.

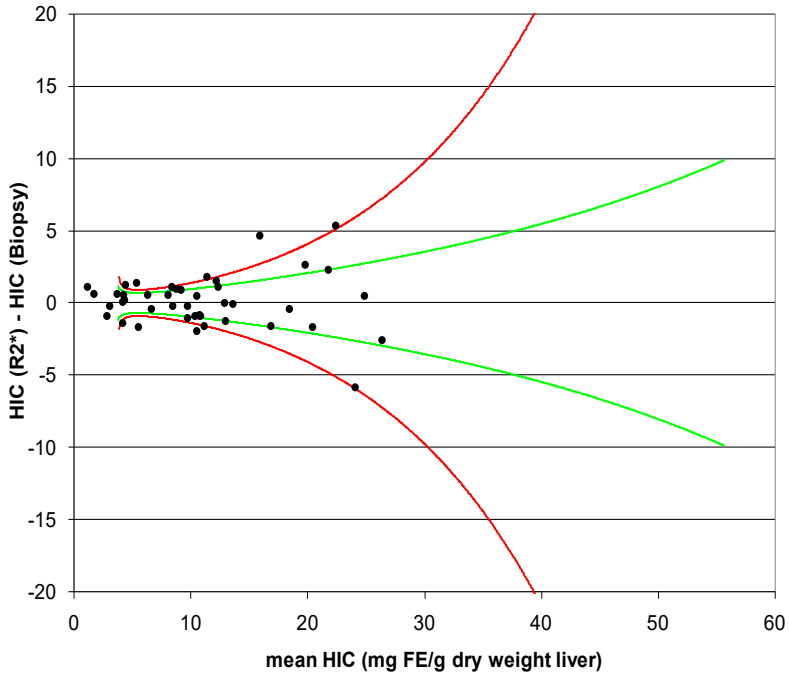

Figure 2: T2*-fit precision model curves overlaid on the Bland-Altman analysis of the MRIRON study data (Fig. 1B). The red curves (outer lines) represent the 95% confidence interval computed via numerical simulation of the fit precision, as described in the text. Simulation parameters matched experimental conditions ($TE_1 = 1.1$ ms, SNR 50, $n = 20$). The error obtained by simulation follows the trend of the experimental error. The green curves (inner lines) represent the simulated 95% confidence interval of a R2*-UTE experiment ($TE_1 = 0.05$ ms, SNR 50, $n = 21$) with otherwise identical experimental parameters as R2*-GRE. The much narrower flare suggests significantly higher prediction accuracy for high and massively overloaded patients, e.g., at 20 mg Fe/g UTE outperforms R2*-MRI by a factor of 2, at 40 mg Fe/g by a factor of 4, and by higher factors at higher HIC values.

2.8 Rationale

The novel R2*-UTE technique will enable accurate quantification of hepatic iron in patients with massive iron deposits in the liver, thereby providing an accurate means to diagnose, monitor, and accordingly administer iron-unloading treatments in this group, for whom the only reliable method for HIC quantitation available currently is invasive liver biopsy. This new technology

will impact the care of many different populations with iron overload, such as patients with SCD and TM. There are presently 100,000 people with SCD and 1,000 with TM in the United States, but approximately 330,000 babies are born every year with one of these diseases worldwide.[63] Blood transfusions frequently need to be given either intermittently or chronically to these 2 groups. With the recent evidence that transcranial Doppler ultrasound screening can significantly decrease the incidence of primary stroke in SCD,[64] an increasing number of programs in the United States and worldwide are using this screening modality and intensifying the use of chronic erythrocyte transfusion. The implementation of a regular program of chronic transfusions has significantly impacted survival of patients with TM, another group for which blood transfusions are crucial.[1] Patients with other disorders such as DBA, sideroblastic anemia and other myelodysplastic syndromes, severe aplastic anemia, congenital dyserythropoietic anemia, severe forms of enzyme-deficient hemolytic anemias (e.g., pyruvate kinase deficiency) or red blood cell membrane defect disorder (e.g., hereditary spherocytosis), and hereditary hemochromatosis collectively comprise a significant fraction of additional patients who will benefit from iron quantification via UTE technology.

With the improvement in the survival rates of patients with different malignancies, the number of cancer survivors has fortunately grown at a very fast rate in recent years. Frequently, the volume of blood usage during intense chemotherapy regimens is high enough to impose an iron burden, especially among those who undergo bone marrow transplantation. With marked improvements in the success rates of cancer treatment, body iron assessment becomes an important part of the long-term care of cancer survivors. Hence, noninvasive methods for iron assessment are very attractive for optimal management of cancer too, and can have a much larger impact than when restricted to benign hematologic conditions only.

The accurate and noninvasive method for body iron assessment using R2*-UTE will significantly advance the field by improving the care of all populations affected by iron overload and providing a reliable means of quantifying iron over a large range of possible HIC values, especially very high ones. Accurate iron quantification at high tissue iron concentrations will allow better clinical decisions to be made regarding the use of intensified and more aggressive chelation therapies. For example, higher than usual doses of iron chelators, continuous IV use of desferal, and iron chelation combination therapy can be administered more safely if accurate liver iron estimations are available. Since MRI is a safe technique, noninvasive and accurate measurements of HIC can be taken repeatedly without entailing the risks encountered during liver biopsy. The use of R2*-UTE for iron estimation will allow administration of intensified and optimized iron chelation therapy in heavily iron-overloaded patients without exposing them to unnecessarily high doses or long periods of treatment, thereby reducing treatment toxicity.

An additional important consideration is the cost of liver biopsy versus that of MRI-based quantitation of iron. Liver biopsy for HIC quantification is expensive, because it includes costs of anesthesia, interventional radiologist or surgeon fees, and hospital and laboratory fees. A liver biopsy can on average cost more than $4000; in contrast, an MRI for liver iron quantitation costs approximately $500. Furthermore, MRIs provide faster results, as opposed to the 48–72 h turnaround time for liver biopsies.

Compared to R2-MRI measurements that are based the acquisition of spin echo signals, R2*-MRI based on gradient echo signal acquisitions is a faster, safer, and cost-efficient technique.

Such R2*-sequences can principally be played out within a single breath-hold maneuver so that an entire R2*-MRI measurement could be accomplished within about 5 minutes after some initial localizer images. In contrast, an R2-MRI measurement takes about 20 min or more, and this long duration may necessitate the use of general anesthesia in young patients. Also, evaluation of the only generally accepted R2-quantification method requires data analysis in a central laboratory in Australia, which adds to cost and turnaround time.

Most centers in the country and worldwide are adopting stronger magnetic field MRI machines, such as 3T MRI or higher. St. Jude owns two 3T MR and two 1.5T magnets. It is conceivable that in the next decade the current 1.5T MRI systems will be outdated and would be substituted by stronger field strength MRI machines. The MIDAS study will allow not only the testing of a new technique, which will be more precise for quantification of tissues with high iron concentrations, but will also anticipate the future trend in utilization of stronger field strength MRI machines at the same time as defining the best magnetic field strength for tissue iron quantification.

Finally, faster and more frequent HIC assessment in cases of massive iron overload may improve patient compliance to treatment, which may result in better responses to iron-overload treatment.

In summary, an accurate method that allows tissue iron quantification at very high HIC values will allow clinicians to optimize iron overload therapy, which will likely improve patients' quality of life by reducing disease- and treatment-associated morbidity. Adherence of patients to treatment can be improved with better treatment monitoring and costs reduced by using noninvasive methods. Once calibrated, R2*-UTE can be translated to clinical use, offering a good opportunity to increase the reach and impact of this new technology.

3.0 RESEARCH PARTICIPANT ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

3.1 Inclusion Criteria

3.1.1 Patients

- History of 12 or more lifetime erythrocyte transfusions, and
- Need for liver iron content assessment(by MRI or liver biopsy)

3.1.2 Healthy volunteers

- Age ≥ 18 years, and
- Willingness to undergo MRI testing for investigation and implementation of the R2*-UTE technique

3.2 Exclusion Criteria

3.2.1 Patients

- Presence of certain MR-unsafe foreign material in the body, or other conditions that make the research participant ineligible for an MRI scan per St. Jude policies.

- Any condition or chronic illness that in the opinion of the PIs makes participation on study ill-advised.

3.2.2 Healthy volunteers

- History of blood transfusions in the lifetime

- Known history of hereditary hemochromatosis

- Presence of certain foreign, MR-unsafe material in the body, or other conditions that make the volunteer ineligible for an MRI scan per St. Jude policies.

- Any condition or chronic illness that in the opinion of the PIs makes participation on study ill-advised.

3.3 Research Participant Recruitment and Screening

There are approximately 200 patients within the St. Jude Network (St. Jude Children's Research Hospital, St. Jude Domestic Affiliates, and the adult Hematology and Oncology program at the University of Tennessee Health Sciences Center) who have a history of iron overload due to multiple erythrocyte transfusions and need annual HIC assessment. Participants will be recruited from St. JudeHospital, St. Jude Affiliates, and the University of Tennessee Health Sciences Center. Participants will be approached during regular clinic visits by the study clinicians or their designees according to study eligibility criteria. Consenting for the study will take place at St. Jude. This study will be approved by St. Jude and University of Tennessee's Institutional Review Boards, and all study participants (or their legal guardian, if research participant is a minor) will provide a signed informed consent.

Eligible adult patients will be referred to the MIDAS study staff by the adult hematologist/oncologist or his staff. Potential candidates will be provided with the study brochure and instructed to contact the MIDAS investigators if interested in participating. The MIDAS investigators will then schedule a research visit at St. Jude where they will be consented and will have all his research activities performed.

3.4 Enrollment on Study

A member of the study team will confirm potential participant eligibility as defined in Section 3.1-3.2, complete and sign the 'Participant Eligibility Checklist'. The study team will enter the

eligibility checklist information into the Patient Protocol Manager (PPM) system. Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The signed consent/assent form must be faxed or emailed to the CPDMO at 595-6265 in order to complete the enrollment.

Enrollment will be reached and stopped when the total necessary number of liver biopsies (as stated in Section 9.1) is reached in order to allow the analysis of the primary objective.

4.0 STUDY DESIGN AND METHODS

4.1 Study Design

The MIDAS study is a prospective and non-therapeutic study that will test a new MRI technique for the assessment of iron overload in the liver: the newly developed R2*-UTE. The R2*-UTE technique, developed by St. Jude investigators from the Department of Radiological Sciences, will be first tested in healthy volunteers for feasibility and implementation of the technique. The technique will then be tested in research participants, who will have both the R2*-GRE and the R2*-UTE techniques performed, in addition to a liver biopsy for liver iron quantitation if clinically indicated. Healthy volunteers will *not* have a liver biopsy performed but only the two types of R2*-MRI techniques will be performed and compared.

Volunteer measurements will be essential to implement the technique before it is tested in patient subjects. We anticipate needing 15 volunteers for initial sequence testing purposes. Since sequence programming is an iterative process, the exact number of needed volunteers may vary, since not all volunteer data will be statistically evaluated. An additional 20 more volunteers will be needed to test advanced sequence types for non-breath hold exams and to compare them to the standard techniques. A subgroup of these volunteers will receive multiple MR scans to test consistency and repeatability of the developed techniques.

4.2 R2*-GRE and R2*-UTE MRI

Study participants will undergo an MR examination of the liver on a 1.5T MRI and a 3T MRI scanner each. Multi-echo GRE sequences will be used to acquire images with increasing TEs. Images of the liver will be obtained in transversal slice orientation through the center of the liver at the level of the origin of the main portal vein. At equivalent slice locations R2*-UTE scans will be performed. As siderosis is a potential confounder of accurateR2* quantification as well as the inability of a sedated patient to hold the breath, R2*-UTE measurements will be repeated with different parameter settings and acquisition modes (such as multiple 2D-or3D-acquisition or fat-suppression) total or the R2*-UTE measurement process to individual research participants who for example have non-alcoholic fatty liver disease in addition to iron overload.[65]

Images and raw data will be transferred to a computer workstation for post processing, including T2* determination and region of interest (ROI) analysis. Quantitative T2* maps will be calculated offline using custom-written programs. ROIs will be drawn either on source images or T2* maps in a homogeneous area of the right hepatic lobe, avoiding blood vessels and obvious bile ducts. To maintain the highest level of comparability, the same ROI will be used for the techniques to be compared, if possible. Raw data transfer allows using alternative post

processing techniques that can lead to a better R2* fit such as phase sensitive evaluation which may better quantify the contribution of the noise in the MR measurement, or the use of autoregressive–moving-average (ARMA) modeling which yields additional fitting parameters that can be used to increase the trust of the fit or calculate/extract for example confounding fat contributions.

Multiple reviewers from the Departments of Radiological Sciences or Hematology (e.g., clinical research assistants, translational imaging scientists, or radiologists) will be trained to process T2* maps. These reviewers will be trained to place ROIs in the liver by the radiologists or physicists participating in the study. All examiners will be blinded to the liver biopsy results and clinical information.

4.3 Liver biopsy

Indications for liver biopsy include, but are not limited, to the need to quantify liver tissue iron and the need to obtain histopathological information of the liver tissue. Liver biopsies will only be performed if clinically indicated. Liver biopsy procedures will be performed by an interventional radiologist at St. Jude Hospital for subjects under the age of 26. Biopsies for subjects 26 years of age and older will occur at an adult facility (Regional One Health, or other adult hospital). The technique to be used is coaxial percutaneous (transcapsular) technique; however, a coaxial transjugular technique may be performed in subjects with increased bleeding diathesis, since it is associated with less hemorrhagic risk. Subjects will undergo a pre-assessment visit with the physician performing the procedure, during which the risk of bleeding will be evaluated through coagulation tests and platelet count (Table 2) performed within 48 h of the procedure. An informed consent for the procedure will be signed by subjects 18 years or older or by the legal guardian if the subject is a minor. Risks related to the coaxial percutaneous (bleeding and sclerosis of the tract) and coaxial transjugular (bleeding, carotid artery damage, arrhythmia, air embolus, and hepatic arterial venous fistula) techniques will be discussed during the informed consent process. Subjects will be required to be fasting and will be sedated for the procedure. The coaxial percutaneous biopsy technique uses a size-appropriate needle sheath that passes through the liver capsule only once. Passes are then taken through the sheath in the interior of the liver. The coaxial transjugular technique uses a sheath in the hepatic vein and passes are taken through the sheath. After the procedure, subjects will be observed and their vital signs and hematocrit monitored. Subjects will be discharged at the end of the observation period in case there are no complications. If there are signs of hemorrhage or significant pain, the subject will be admitted and treated accordingly. At least 5 mm of liver tissue will be placed in 10% formalin and sent for a pathology review at St. Jude for histology, iron stain, fibrosis, and fat content evaluation. At least 5 mm of liver tissue will be placed in a metal-free specimen vial, which will be refrigerated and mailed to Mayo Clinic Laboratory (Rochester, MN) for quantitation of liver tissue iron.

Because St. Jude is a pediatric hospital it will be required that any complication arising from a liver biopsy procedure in a subject older than 21 years be managed at adult facilities. Possible complications from liver biopsies include abdominal pain, hepatic bleeding, and biopsy site infection. The risk of complication with ultrasound-guided liver biopsies is very small, however.

In the prior MRIRON protocol, which also included adult participants, there were 7 out of 50 liver biopsies (14%) that required overnight observation for pain. All episodes completely resolved with no sequelae. There were no cases of infection or hepatic bleeding. As a precaution, the following plan will be followed for subjects ≥ 21 years who undergo liver biopsies at St. Jude:

- If an adult (someone older than 21 years) needs to be admitted for pain/observation, he/she will be admitted to the Hematology service at St. Jude.
- The ICU attending will be informed of any adult research participants admitted to St. Jude following a liver biopsy under the MIDAS study.
- If an adult (someone older than 21 years) needs an ICU admission, transportation to an adult facility after the research participant has been stabilized will be arranged. St. Jude will not be responsible for the medical costs related to the admission in the adult facility, but will provide transportation to the adult facility and will ensure that the patient is medically stable for the transfer. Adult facilities include the main adult hospitals in Memphis, such as Methodist family hospitals, Baptist Hospitals, St. Francis, and the Delta Medical Center. The choice of adult facility will depend on the adult participant's network insurance coverage and primary clinicians' preference, which will all be verified prior to the transfer. The primary treating clinician of the adult patient will be communicated prior to the transfer, and will assume their care, once the transfer to the adult facility is completed.

The plan above was reviewed and approved by the Anesthesiology Chair, St. Jude Clinical Director, Sedation Nurse Coordinator, Intervention Radiology, ICU Medical Director, ACU Nursing Director, and Inpatient participant Nursing Director.

5.0 REQUIRED EVALUATIONS, STUDY INTERVENTION, AND OBSERVATIONS

All studies in MIDAS will be performed within 30 days of each other. All study participants' medical history will be ascertained and will include all pertinent medical history including, but not limited to, transfusion history, menstrual history, history of endocrinopathies and cardiac disease, medications used including chelation therapy, etc.

5.1 Study Evaluations

Table 2: List of study tests for the MIDAS study.

A.	Pregnancy test prior to undergoing MRI examination *(for female research participants, including both healthy volunteer and patients, of child-bearing potential only)*
B.	Complete blood count (CBC), Serum ferritin, transferrin saturation, total iron binding capacity, and an extra aliquot of blood for storage[1]
C.	Activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, platelet function analyzer (PFA-100)[2]
D.	Liver biopsy with determination of liver iron concentration and histology[3]

E. 1.5T and 3T MRI exams of the liver

[1] An extra aliquot of blood (5cc) will be drawn and stored for future research

[2]Pre-liver biopsy lab work, performed within 48 h of procedure.

[3] Performed within 30 days of the MRI tests and only in research participants who have a clinical need for liver biopsy as determined by the treating clinician.

Currently, all iron-overloaded research patients undergo annual R2*-GRE exams (or more frequently if clinically indicated) for clinical monitoring. All iron-overloaded patients will be approached to participate in MIDAS at the time of their regular clinic visit.

All study participants will undergo a 1.5T R2*-GRE and 1.5T R2*-UTE study to evaluate their clinical need for a liver biopsy (Figure 3) In most cases, MRI examination will precede the liver biopsy. In some cases, however, due to occasional clinical urgency in obtaining the liver biopsy study, the liver biopsy will be done prior to MRI exams. In these cases, 1.5T R2*-GRE and 1.5T R2*-UTE MRIs will be done within 30 days following the liver biopsy. In addition, a 3T MRI exams consisting of a R2*-GRE and R2*-UTE will be performed.

If an exam based on a 1.5T R2*-GRE measurement that was analyzed using clinical criteria as established in the AIM09 clinical practice protocol fails according to established criteria (failed or non-converging fit precision in more than 10% of the voxel prescribed in the ROI), a liver biopsy is clinically indicated and will be performed (if recommended by the treating clinician) within 30 days of the MRI exam. In other words, in this case, the current R2*-GRE method fails to provide a predicted HIC value because not enough pixels in the selected ROI capture the MR signal decay. In our experience, the insufficient fit corresponds to increased concentrations or iron in the hepatic tissue, and the threshold at which failed fits are seen in individual pixels begins at about 500 Hz and accentuates above 900 Hz, which corresponds to an HIC of ~ 15 and 25 mg Fe/g, respectively.

Healthy volunteers will undergo R2*-UTE and R2*-GRE studies at both, 1.5 and 3T MRI field strengths. No blood work or liver biopsy will be performed on healthy volunteers.

The result of the liver R2*MRI will be shared with the participants' primary clinician and the decision to pursue a liver biopsy will be made by the treating primary clinician. If the participant's primary clinician is from an outside institution (such as in the case of the adult participants), the MIDAS study staff will contact the primary treating clinician and inform him of the MRI results, and a copy of the report will also be mailed or faxed to his office. The primary clinician will review the MRI results and will communicate back with the MIDAS investigators to inform if a liver biopsy is warranted or not. If a liver biopsy is indeed indicated and requested by the treating clinician, an appointment will be scheduled to perform the liver biopsy. Biopsies needed for subjects 26 years of age and older will occur at an adult facility (Regional One Health or other adult hospital), patients 25 or younger will have their liver biopsies performed at St. Jude.

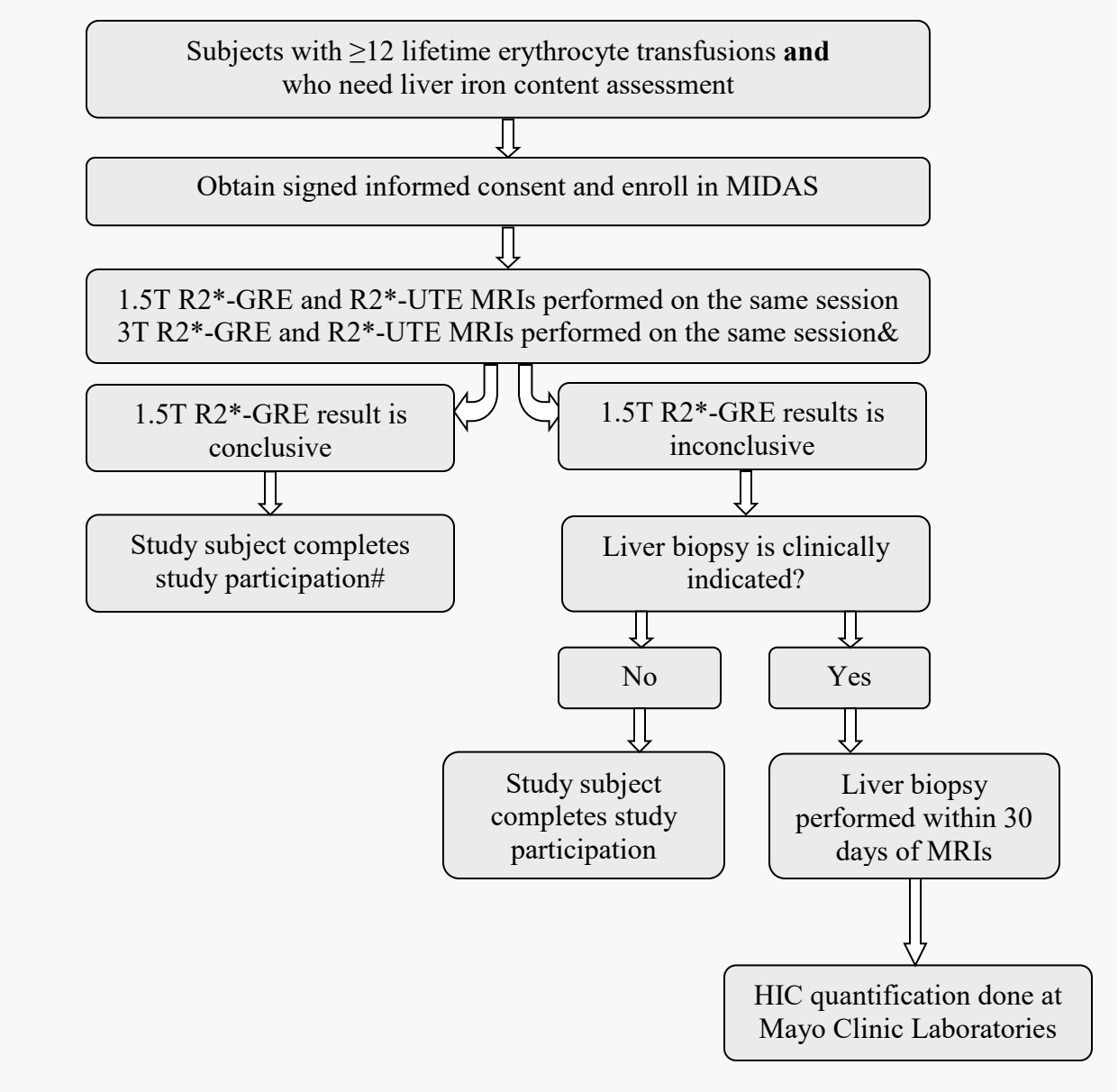

Figure 3: Flow diagram for the screening and testing of subjects in the MIDAS study.
Notes: & if participant requires general sedation for MRI, 3T R2*-GRE and R2*-UTE MRIs will not be performed; # participant may still receive a liver biopsy upon treating clinician's discretion. In most cases, MRI examination will precede the liver biopsy. In some cases, however, due to occasional clinical urgency in obtaining the liver biopsy study, the liver biopsy

will be done prior to MRI exams. In these cases, 1.5T R2*-GRE and 1.5T R2*-UTE MRIs will be done within 30 days following the liver biopsy.

Sample Storage

To enhance the scope of research that can be conducted within the MIDAS study, we will establish a biologic repository consisting of stored blood (DNA, serum phase) from all MIDAS participants. An extra aliquot of blood (5cc) will be obtained and consent provided for the collection, storage and future utilization for research investigations through a currently approved tissue bank protocol. Potential investigations utilizing the biologic specimens include, but are not limited to, more extensive laboratory analyses of biomarkers of iron metabolism and iron overload-predisposing genetic mutations. Accession of samples for future research will require approval of the planned research by the Tissue Resource Committee and the Institutional Review Board. In addition to the protocol-specific testing of specimens, study participants will be offered the option of allowing any leftover serum, or blood cells to be saved or shared for future analyses. Leftover serum or blood cells from subjects enrolled in the MIDAS protocol will be de-identified prior to storage or any future testing and/or sharing and will be kept indefinitely in Tissue Resources at St. Jude.

5.2 Off-Study Evaluations

Subjects will be considered off-study 10 days after all study tests have been completed. No off-study evaluations will be required.

5.3 Long-Term Follow-up Evaluations

Not applicable.

5.4 Staff Training and Integrity of Study Procedures

Radiological staff will be trained to appropriately draw ROIs on R2*MRI maps or T2*MRI source images. The software developed by St. Jude physicists is very interactive and easy to use. Staff will undergo training to perform this test and quality control assessment of their ROI measurements will be performed on a regular basis.

6.0 CRITERIA FOR REMOVAL FROM PROTOCOL AND OFF-STUDY CRITERIA

Subjects will be removed from the study if intolerance to performing the MRI study precludes its completion. Subjects will also be removed from protocol at the subject and/or parents request.

7.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

MRI does not involve radiation, therefore there are no risks associated with radiation injury. As reiterated in a recent report,[66] non-sedate MRI generally is considered a minimal risk study. Female participants (both patients with iron overload and healthy volunteers) of child bearing potential, however will be required a negative pregnancy test prior to undergoing an MRI exam. Study participants may need to be sedated for the MRI exam, which may expose the subjects to

the risks involved with anesthetics and airway complication. In the event that a research participant requires sedation with general anesthesia, only the 1.5T MRIs will be performed. The 3T MRI scan will be entirely omitted due to the risks of an additional sedation for a research study.

R2*MRI is done on a regular basis and the additional R2*UTE exams that will be offered in MIDAS will be an additional 30 minutes of MRI table time at 1.5T. The total time of the additional 3T measurement is about 1 hour table time (including scout imaging and the R2*-GRE). Subjects may experience anxiety and claustrophobia during MRI exams, however. If this occurs, the study will be temporarily interrupted, and the subject will be given instructions on how to best tolerate the exam and offered to watch movie or listen to music to help with the relaxation process during the test. In addition, St. Jude Child Life Services have worked very closely with our pediatric subjects and have helped by offering distraction techniques which have improved the tolerance to not only MRI tests, but many other imaging studies. If even after all these measures the study subject is still not able to tolerate the MRI study, the study will be interrupted.

Liver biopsy is an invasive procedure that may involve risks such as pain, bleeding, and infection. Liver biopsy data will be collected only in research participants who are already undergoing a liver biopsy for clinical indications, as recommended by the treating clinician. Liver biopsy is not a condition for participation in the MIDAS study, and therefore HIC quantitation by liver biopsy will be obtained *only* for subjects with a clinical indication for this test. Research participants can undergo the MRI or a liver biopsy initially, and in research participants in whom the noninvasive method (with MRI) is done first and failed to provide a HIC measurement and it is necessary to obtain this information for proper clinical management, a liver biopsy might be recommended by the treating clinician.

7.1 Reporting Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening (i.e., an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly birth defect.
- In the opinion of the investigator, important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above and

may be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Fatal or Life-Threatening Adverse Events

A fatal adverse event will precipitate the interruption of the study. Permanent closure of the study will be evaluated by the SJCRH IRB.

7.2 IRB Notification by Investigator

In addition to the continuing review reports to the IRB, the Principal Investigator is responsible for reporting all serious or unexpected adverse events that impact the safety of or risk to the study subjects. Unexpected death will be reported to the IRB office immediately. Serious, unexpected events are to be reported within 48 hours, and all others within 10 working days.

7.3 Recording Adverse Events and Serious Adverse Events

Adverse events will be recorded by study investigators in the adverse events form and a copy will be kept in the study file. Participants will remain on study for 10 days following the last study procedure. This will allow for capture of information in the event that a procedure-related adverse event occurs. All adverse events will be documented in the study database. In the event that a subject has an unresolved event at the time he/she is taken off study, for example, infection, he/she will still be taken off-study and the subject will be followed by the attending physician until resolution of the event. Subjects will be assessed for AE's through communication with their respective attending physicians and chart review.

This study will utilize the CTCAE Version 4.0 for toxicity and performance reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). Additionally, the toxicities are to be reported on the appropriate data collection forms.

7.4 Definitions

7.4.1 Definitions of Adverse Events

The following definitions have been adapted from the 2010 FDA reporting regulations and International Conference on Harmonisation (ICH) guidelines for use in this study:

i. Adverse Event (AE): An adverse event (AE) is defined as any reaction, side effect, or untoward medical event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

ii. Suspected Adverse Reaction: Suspected adverse reaction means any adverse event for which there is a *reasonable possibility* that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. For this study, a suspected adverse reaction will be any event reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation that are thought to be related to the MRI exam or liver biopsy.

iii. Expected adverse event: Several complications are expected to happen in subjects with hematologic conditions (such as hemolytic anemia, painful crisis, etc.) MIDAS expected adverse events are listed in Table 3 and 4.

v. Unexpected adverse event or unexpected suspected adverse reaction: A MIDAS unexpected AE or unexpected suspected adverse reaction is any AE or SAR that is not a MIDAS expected AE or SAR (listed in Table 3) or not listed at the specificity or severity that has been observed.

Table 3. List of MIDAS expected and potentially serious adverse events in all subjects.

Hematologic Disease Symptoms and Associated Conditions		
Abnormal TCD velocities	Hand-foot syndrome/dactylitis	
Acute chest syndrome	Hematuria	Pain, sternal or rib
Albuminuria	Hemiplegia	Priapism
Amenorrhea	Hemolysis	Proteinuria
Anemia (severe)	Hemorrhagic Stroke	
Aplastic crisis	Hepatic sequestration	Pneumonia
Arthralgia	Hepatomegaly	Pulmonary embolism
Avascular necrosis of hip/shoulder		Pulmonary hypertension
Bacteremia	Hyperbilirubinemia	Pulmonary infiltrate on chest x-ray
Bone infarction	Hypersplenism	Pyelonephritis
Cardiac arrythmia	Hypertension	Renal failure
Cardiomegaly	Hypocalcemia	Renal insufficiency
Cerebrovascular accident	Hyposthenuria	Renal papillary necrosis
Cholecystitis	Hypotension	Reticulocytopenia
Cholelithiasis	Hypoxemia ($PO_2 < 65$mm Hg)	Reticulocytosis (10%–20%)
Cognitive dysfunction	Ileus	Retinopathy
Constipation	Infection, other bacterial	Retinal hemorrhage
Cranial nerve palsy	Infection, pneumococcal	Rhabdomyolysis
Death	Infection, line	Seizure
Decreased renal function	Infection, viral	Septicemia
Decreased lung function	Jaundice	Silent infarct
Delayed growth/puberty	Leukocytosis	Skin ulcer
Depression	Meningitis	Splenic sequestration
Dizziness	Nephropathy	Splenomegaly
Electrolyte imbalance	Osteomyelitis	TCD velocity, transient increase or decrease, or TCD alert
Elevated urinary urobilinogen	Pain, back	TIA
Elevated serum transaminases	Pain, chest	Transfusion, unanticipated
Elevated TCD velocities	Pain, joint	Vaso-occlusive pain
Fever	Pain, long bone	

7.4.2 Adverse Event/Suspected Adverse Reaction Severity Grades

MIDAS uses the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0, for detailed descriptions of Severity Grades. The CTCAE is classified by body system and AE and provides descriptions of events that qualify under each severity rating.

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate daily activities and tasks.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activity of daily living (ADL)**.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Grade 1 AE/SARs are not entered in the electronic CRF or captured in the MIDAS database.

7.4.4 Adverse Event Outcome

The terms used to describe AE outcome are as follows:

Ongoing
Resolved without sequelae
Resolved with sequelae

8.0 DATA COLLECTION AND CONFIDENTIALITY

8.1 Data Collection

Data collection and data management for this investigation will be conducted through the SJCRH Hematology Department. Co-investigators and CRAs assigned to this protocol will be responsible for assisting the PI in assuring protocol compliance as well as reviewing, transcribing and tracking of all clinical and safety related data.

Clinical data will be transcribed from source documents to the study database. This ensures that all protocol required data elements are captured. A database will be created for this study by the Clinical Research Informatics Division. Data will be entered by a Clinical Research Associate (CRA).

8.2 Study Monitoring

The Principal Investigator and study team are responsible for ensuring protocol compliance. The study team will hold quarterly team meetings and review case histories or quality summaries on participants.

Source document verification of eligibility and the informed consent process for 100% of St. Jude participants will be performed by The Eligibility Coordinators.

The Clinical Research Monitors will review up to 10% of the study participants annually for appropriateness of the informed consent process, eligibility, SAE reporting (TRACKS), and patient status. Additional information may be monitored at the request of the IMC, the IRB, or other institutional administration. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC).

Continuing reviews by the IRB and CT-SRC will occur at least annually. In addition, SAE reports in TRACKS (Total Research and Knowledge System) are reviewed in a timely manner by the IRB/ OHSP.

8.3 Confidentiality

Records from the study which identify the study participant will be kept confidential in a password-protected database. Access to study database will be allowed only to study investigators and designated CRAs. Subject identifiers will be removed from any published material.

9.0 STATISTICAL CONSIDERATIONS

This is a non-therapeutic, prospective study aimed to develop a novel R2*-UTE imaging sequence technique to non-invasively and accurately estimate high hepatic iron content (HIC) values (≥25mg Fe/g). The current standard management procedure for patients at risk of iron overload at St. Jude is to estimate their (HIC) by the calibrated R2*-GRE imaging technique, which becomes unreliable when HIC is greater than 25 mg Fe/g. Therefore, the success of the study will provide better monitoring and guidance to the clinical management of patients with massive iron overload.

The primary objective is to test the association of hepatic iron content (HIC) measured with the newly developed 1.5T R2*-UTE technique and HIC quantified by liver biopsy in subjects with iron overload. The liver biopsies will be obtained as indicated by good clinical management practice, and it is expected that most research participants who will receive the liver biopsy will have an inconclusive result by the R2*-GRE method. The iron quantification assessed in liver biopsies will be correlated with the R2*-UTE signals. If the correlation is sufficiently high, then a statistical model relating HIC by R2*-UTE and liver biopsy values will be established, as was done for R2*-GRE.[32]

9.1 Power and Accrual

Based on MRIRON study, we found that the correlation between R2*-GRE and HIC is 0.56 for patients with HIC>15mg Fe/g. The new R2*-UTE technique is expected to perform better for measuring high HIC values. Therefore, instead of testing if the r_{UTE} is different from zero, here we wish to test if the r_{UTE} is greater than 0.6. The one-sided testing hypotheses can be formulated as below: the null hypothesis H_0: $r_{UTE} \leq 0.6$ against the alternative hypothesis H_a: $r_{UTE} \geq 0.6$ we assume the true r_{UTE} to be 0.8. The study will be designed to have a significance level at $\alpha = 0.05$ and power at $1-\beta = 0.8$. Although the parameter space for H_0 is large, to estimate the required sample size, we only need to consider the scenario that requires the largest sample size, which is: H_0: $r_{UTE} = 0.6$ v.s. $r_{UTE} = 0.8$. Based on a one-sided test, this design requires **41** liver biopsy samples.

We hope to accrue about 10-12 liver biopsies per year, so it will take approximately 4 years to achieve the desired sample size. Study subjects will be allowed to participate on the study only once in order to avoid potential bias introduced by repeat measurements on the same patient. There is a small chance that the liver biopsy specimen may be deemed inadequate due to technical sampling reasons. In this case, we will amend the protocol and continue patient accrual until we have obtained 41 evaluable liver biopsy specimens.

Approximately 20% of patients with iron overload have HIC values above 25 mg Fe/g, placing them in the category of massive iron overload. Because we aim at obtaining 41 liver biopsies for the analysis of the primary objective, we estimate that approximately 200 patients will undergo MRI testing with both R2*-GRE and R2*-UTE.

Healthy volunteers will be necessary for sequence development, and later for testing the robustness and repeatability of the UTE sequences. We estimate that approximately 35 healthy volunteers will be needed for sequence development and implementation.

9.2 Statistical Analysis

For the primary objective: *To explore the association of hepatic iron content (HIC) measured with the newly developed 1.5T R2*-UTE technique and HIC quantified by liver biopsy in subjects with iron overload.*

The relationship of HIC measured with 1.5T R2*-UTE and quantified by liver biopsy in subjects with iron overload will be explored graphically. If the linear relationship exists, the Pearson's correlation between the HIC measured with 1.5T R2*-UTE technique and the HIC quantified by liver biopsy will be calculated and its 95% confidence interval (CI) will also be reported. If the correlation is sufficiently high, i.e., the lower bound of the 95% CI exceeds 0.7, a simple linear regression model will be established for the HIC prediction. The 95% prediction bounds will be established. If more complicated pattern shown graphically, appropriate model will be developed to describe the relationship.

For the secondary objectives: *To explore the relationship 1) between 1.5T R2*-UTE and 1.5T R2*-GRE measurements in healthy volunteers and in subjects with iron overload and 2) between 1.5T R2*-UTE measurements with iron studies (serum iron and transferrin saturation) in subjects with iron overload.* Analyses will be implemented similarly as the analyses planned in the primary objective to explore the relationships. Pearson's correlation will be used if there is graphical evidence of linear relationship, similarly to the Primary Objective. 95% confidence interval (CI) will also be reported. If correlation is sufficiently high, a simple linear regression model will be developed and the 95% prediction bounds will be established. If more complicated pattern shown graphically, appropriate model will be developed to describe the relationship.

Objectives 1.3.1, 1.3.2, 1.3.3, 1.3.4, and 1.3.5 were classified as exploratory since they do not directly support the future implementation of the 1.5 R2*-UTE technique, and the gathering of the information from variables within these objectives is not yet clear in the near future but may ultimately generate additional hypotheses for future investigation. The purpose of the exploratory objectives is to (1) evaluate how well the R2*-UTE and the R2*-GRE signals correlate in subjects where the R2*-GRE does not fail. The analyses will provide the evidence of whether the R2*-UTE technique can replace the R2*-GRE technique even in the lower HIC

cases. (2) understand the relationships among a set of variables measured, Similar analyses will be implemented similarly to the analyses implemented in the primary objective.

10.0 OBTAINING INFORMED CONSENT

The informed consent will be obtained by one of the study investigators or his designee in the presence of at least one witness. Subjects identified as eligible for the study will be invited to participate. Although subjects may be recruited from the Adult Program or St. Jude Affiliates, *all participants will be consented in one of the St. Jude Hospital Outpatient Clinics*. A translator will be provided if necessary. The subject and a legal guardian will be introduced to the study. Objectives and procedures required during the study will be discussed with the family and subject, while reading the informed consent. Subjects older than 18 years-old will sign their own informed consent statement. The legal guardian will sign the informed consent if the subject is a minor. Verbal assent will be obtained from subjects 7 to 14 years of age and written assent from subjects 14 to 17 years old.

In addition, during the development of the R2*-UTE technique, healthy volunteers will be needed to implement and test the new R2*-UTE technique. Healthy volunteers will be recruited among St. Jude employees and will also sign an informed consent for MIDAS, and will have their MRI result data added to the study database. Both R2*-GRE and R2*-UTE using both 1.5T and 3.0T MRI field strength testing will be performed in volunteers in order to proper test and implement the technique in both MRI field strengths.

11.0 SUBJECT COMPENSATION

Study participants (patients only) will be given $75.00 per study visit (for a possible maximum amount of $225) as partial compensation for transportation and time commitment. This sum will be given to study participants at the completion of each study visit. No compensation will be given to healthy volunteers.

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APPENDICES

APPENDIX I:

Schedule of Required Events

Study	Screening	Visit 2
Pregnancy Test *(for female research participants , including both healthy volunteer and patients, of child-bearing potential only)*	x	
1.5T and 3T R2* - GRE MRI 1.5 and 3T R2*- UTE MRI	x	
Complete blood count (CBC), BUN, creatinine, serum ferritin, transferrin saturation, total iron binding capacity, and an extra aliquot of blood for storage	x	
Complete blood count, activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, platelet function analyzer (PFA-100)		x
Liver Biopsy*		x

Note: *When clinically necessary, research participants may undergo the liver biopsy procedure prior to the MRI examinations. This will be determined by the treating clinicians who will judge the clinical urgency in obtaining the liver biopsy. In these cases, the liver MRI exams will be performed within 30 days following the liver biopsy.

Pre-liver biopsy lab work, performed within 48 h of liver biopsy.

Biopsy can be performed within 30 days (after or prior to) the MRI tests and only in research participants who have a clinical need for liver biopsy as determined by the treating clinician.

APPENDIX II:

STANDARD OF CARE (ROUTINE) TESTS

Routine Laboratory Tests
Complete Blood Count
Serum ferritin Transferrin saturation Total Iron Binding Capacity
Activated partial thromboplastin time (aPTT) Prothrombin time (PT) Fibrinogen Platelet function analyzer (PFA-100)

Routine Diagnostic Tests
1.5T and 3T R2* - GRE MRI (liver)

RESEARCH TESTS

Research Laboratory Tests
Pregnancy Test

Research Diagnostic Imaging
1.5 and 3T R2*- UTE MRI (liver)

Blood/Tissue Banking
Leftover serum and blood cells and extra aliquot of blood from the research blood draw and biopsy.