Legacy Biomarker Qualification Project Status Update[1]

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[1] The content you provide in this completed Status Update will be publicly posted as part of the section 507 transparency provisions.

I. Context of Use

A. Biomarker Category

Diagnostic.

B. Intended Use in Drug Development

The indented use in drug development can be stated thus: to identify patients who are more likely to have liver histopathologic findings appropriate for inclusion in non-alcoholic steatohepatitis (NASH) clinical trials.

C. Context of Use Statement

Use Statement

Iron corrected T1 (cT1) relaxation time of liver tissue is a diagnostic enrichment biomarker that can be used, in conjunction with clinical risk factors, to identify patients who are more likely to have liver histopathologic findings appropriate for inclusion in non-alcoholic steatohepatitis (NASH) clinical trials.

Conditions for Qualified Use

General Considerations

- For use in clinical trials for agents which propose to alter (any combination of) the hepatic adiposity, fibrotic- or inflammatory status of liver tissue
- To be used in conjunction with clinical risk factors and/or other diagnostics
- To be used as a pre-screening strategy to select participants more likely to have histopathologic findings
- To be used as a safety consideration with the aim of reducing unnecessary biopsies
- Diagnosis of NASH to be confirmed via histopathology
- During pre-screening, potential participants will undergo an MRI examination to determine whether further evaluation using biopsy is required
- During pre-screening, participants with a cT1 score under the proposed cut-off will be excluded; participants meeting or exceeding the cut-off threshold will be evaluated further using biopsy to determine if the enrolment criteria of the clinical trial had been met

Population Considerations

- Participant population are adults aged 18 or above
- Participant population are those with clinical signs suggesting non-alcoholic fatty liver disease (NAFLD)
- The desired participant population for inclusion in the clinical trial is at-risk participants that meet any of the following criteria:
    - histopathological findings of NAS≥4 and F≥2

- histopathological findings of NAS≥4 and F2-F3 (not F1 and not F4)
- biopsy confirmed NASH

Data Acquisition Considerations

- Modality: Magnetic Resonance Imaging (MRI) at 1.5T or 3T on MRI systems supporting fast T1 mapping
- MRI acquisition protocols as per Measuring Iron Corrected T1 – RA252
- Participants are expected to have fasted (solids and fluids) for at least 4 hours before the MR examination
- Water is allowed in small quantities within 4 hours of the MR examination (i.e. sips)
- Routine MR safety screening applies
- Only for use in participants where MR is not contraindicated
- Use of MR contrast agents is not allowed

Post Processing Considerations

- Iron corrected T1 is computed as per the published algorithm [1]

Biomarker Interpretation

- Iron corrected T1 is a biomarker that quantifies the T1 MR relaxation time of liver tissue and corrects for the local magnetic effects exerted by hepatic iron that artificially shorten the T1 measurement
- Corrected T1 values in the liver have been shown to correlate with both disease activity (inflammation and ballooning) and fibrosis histology [2]
- Iron corrected T1 can be used as a rule-out diagnostic for the purpose of reducing unnecessary biopsy of potential participants that will ultimately not meet the enrolment criteria for clinical trials based on histopathology
- Iron corrected T1 can discriminate between potential participants in an at-risk population, as necessitated by the study design of the clinical trial, based on using the histopathological criteria listed against each grouping as the ground truth reference:

Table 1 - Histopathological criteria for distinguishing between different at-risk populations.

At-Risk Population		Histopathological Criteria
Target Population	Off-Target Population	
NAS≥4 and F≥2	NAS<4 or F<2	NAFLD Activity Score (NAS) CRN and fibrosis staging system [3]
NAS≥4 and F2-F3	NAS<4 or F<2 or F=4	NAFLD Activity Score (NAS) CRN and fibrosis staging system [3]
NASH	Simple Steatosis	NASH diagnosis based on the Brunt system [4] or FLIP algorithm [5]

- A cT1 cut-off of 800ms is proposed with the following performance metrics derived from independent training and validation datasets. Refer to Table 2, Table 3 and Table 4.

Table 2 – Diagnostic performance using a cT1 cut-off of 800ms to discriminate NAS≥4 and F≥2 participants in an at-risk population.

Classification Criteria		
Diagnostic performance metrics using a cT1 cut-off of 800ms to discriminate between: NAS<4 or F<2; and NAS≥4 and F≥2		
Metric	**Training Dataset**	**Validation Dataset**
Classification Function		
Sensitivity	0.91	0.71
Specificity	0.37	0.67
Predictive Value		
NPV	0.83	0.93
PPV	0.55	0.28
Enrichment Analysis		
Enrichment %	20%	82%

Table 3 - Diagnostic performance using a cT1 cut-off of 800ms to discriminate NAS≥4 and F2-F3 participants in an at-risk population.

Classification Criteria		
Diagnostic performance metrics using a cT1 cut-off of 800ms to discriminate between: NAS<4 or F<2 or F=4; and NAS ≥ 4 and F2-F3		
Metric	**Training Dataset**	**Validation Dataset**
Classification Function		
Sensitivity	0.91	0.71
Specificity	0.32	0.67
Predictive Value		
NPV	0.87	0.93
PPV	0.41	0.28
Enrichment Analysis		
Enrichment %	20%	82%

Table 4 - Diagnostic performance using a cT1 cut-off of 800ms to discriminate simple steatosis and NASH in an at-risk population.

Classification Criteria		
Diagnostic performance metrics using a cT1 cut-off of 800ms to discriminate between: simple steatosis and NASH		
Metric	**Training Dataset**	**Validation Dataset**

Classification Function		
Sensitivity	0.87	0.51
Specificity	0.43	0.73
Predictive Value		
NPV	0.65	0.59
PPV	0.73	0.66
Enrichment Analysis		
Enrichment %	15%	29%

Decision Tree

As per the guidance document *Qualification Process for Drug Development Tools*, we include a decision tree diagram to provide additional clarity to the proposed COU for cT1. Figure 1 shows the application of cT1 in the proposed COU and includes the actions that would be taken based on the interpretation of results.

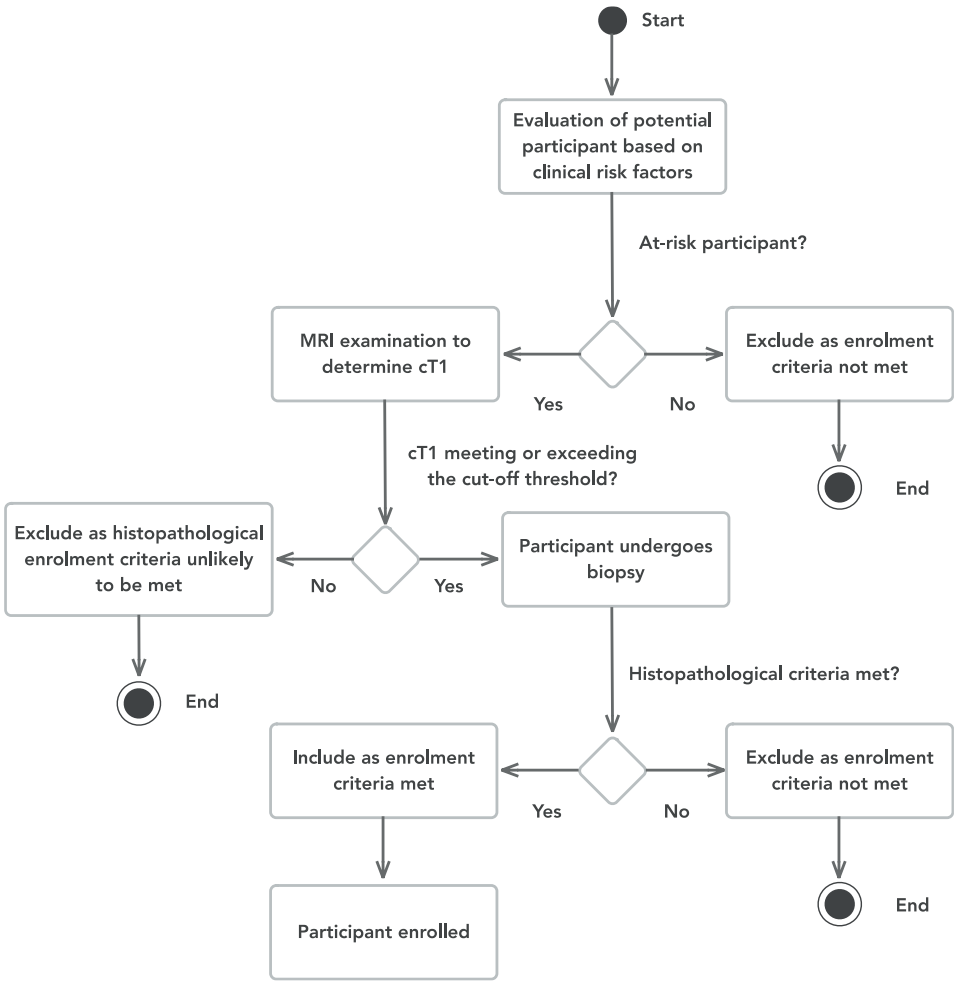

Figure 1 – Decision tree diagram for cT1 COU.

For a complete description of the proposed biomarker, refer to Volume 002: Biomarker Description.

II. Drug Development Need

A. Nature and Extent of Need

As of September 2018, there are 102 ongoing clinical investigations listed on www.clinicaltrials.gov in the United States alone which cite fatty liver disease as the condition under investigation. Despite the high prevalence of steatosis, defined as a liver fat > 5%, recruitment for these trials is inefficient as only a subset of participants with steatosis will have steatohepatitis. Non-alcoholic steatohepatitis (NASH), the more aggressive form of non-alcoholic fatty liver disease (NAFLD), may progress to cirrhosis and hepatocellular carcinoma (HCC). Since NASH is estimated to overtake hepatitis C virus infection as the leading cause of liver transplantation in the US in the coming decade, and there are no current FDA-approved therapies for this disease, the need to find appropriate therapeutic targets is now more urgent than ever before [6]. Prior to enrolment, a biopsy is required to confirm the presence of NASH. However, a significant number of potential participants will not have the pathological hallmarks of NASH (ballooning, inflammation) or fibrosis as confirmed by a histopathological analysis. Clinical trials thus run the risk of failing to recruit sufficient numbers of histologically-eligible subjects to appropriately power the clinical trial to demonstrate that the compound under investigation significantly alters participant pathology. Furthermore, subjects who do not have NASH will have undergone an unnecessary, risky procedure.

B. Proposed Benefits and Risks

The gold standard procedure for the assessment of the degree of fibrosis and the severity of disease activity (inflammation and ballooning) is the liver biopsy. It is invasive, with a definite though small morbidity, and is often not acceptable to patients [7]. This results in poor recruitment rates and can be difficult to justify repeated examinations. The accuracy of liver biopsy for assessing fibrosis and inflammation has been questioned, as it assesses only 0.002% of the liver, and up to 30% of results can be false negatives [8]. It carries a significant risk of serious bleeding complications, which is further amplified as patients with severe liver disease have abnormal coagulation [9, 10].

NASH patients are at risk of developing hepatocellular carcinoma. There is a definite death rate associated with biopsy of the non-tumour liver, which is likely to be higher in tumour biopsies given the increased vascularity of hepatocellular carcinoma (HCC). Furthermore, although the risk of tumour seeding is low (approximately 2%-3% [11]) this has the potential to rapidly transform the prognosis from curable to incurable. A recent review on the topic discusses the need and

value of liver biopsy for clinical and research purposes in HCC patients, as well as the ethical considerations for when to biopsy [11].

Histology itself is imperfect: with a liver biopsy, there is significant intra- and inter-observer variability in histological interpretation [12]. This has led to several different grading and staging systems for liver characterisation, such as Knodell [13], Ishak [14], METAVIR [15], Scheuer [16], Brunt [4] and the NAFLD Activity Score [3]. Agreement between these classifications is limited, even when the same slides are assessed in comparative studies [17].

It is therefore vital that this procedure is performed only on potential participants where the outcome of the biopsy is expected to guide management, and should involve discussion between patient and clinician regarding risk versus benefit [18].

Drug development is not currently benefitting from the many advances in biomedical sciences. It would be more ethical if potential participants were screened prior to enrolment in order to limit unnecessary biopsies and refine the pool of participants towards the intended patient population, i.e. those more likely to have NASH.

Iron corrected T1 (cT1) is a non-invasive, non-composite[2] biomarker that corresponds to the T1 relaxation time of liver tissue, correcting for the effects of hepatic iron content, as iron may result in an underestimation of liver disease by artificially shortening the T1.

In patients with liver abnormalities, correcting for hepatic iron is even more important to mitigate against the risk of potential underestimation of the disease using MRI. Over the last years, there has been accumulating evidence for a strong association between hyperferritinemia and mild iron overload unrelated to hereditary hemochromatosis and manifestations of the metabolic syndrome [19, 20], including NAFLD [21-24]. Increased ferritin levels were detected in about 30% of unselected patients with NAFLD [23, 25-28].

In this submission, we propose the use of a T1 measure that corrects for hepatic iron, cT1, as a rule-out diagnostic for the purpose of reducing unnecessary biopsy of potential participants that will ultimately not meet the enrolment criteria for clinical trials based on histopathology.

Iron corrected T1 is not proposed as a replacement for any biomarker currently in use, but as an additional diagnostic enrichment tool. cT1 is an imaging biomarker that relies on MR and post-processing software, and therefore has a favourable risk/benefit profile.

C. Knowledge Gaps Addressed

[2] Refer to *Qualification Process for Drug Development Tools*.

Within the proposed COU, the cT1 biomarker will enable better enrollment in clinical trials of NASH compounds.

The iron corrected T1 biomarker, if qualified within the proposed COU, will enable sponsors to non-invasively characterise liver tissue and identify potential participants more likely to meet enrollment criteria. This will result in better recruitment rates by reducing the need for unnecessary biopsy of potential participants that will ultimately not meet the enrolment criteria for clinical trials based on histopathology.

See Volume 002, Document 001: Iron Corrected T1 Biomarker Description – RA251 for a more complete description of the proposed biomarker.

D. Biomarker Interpretation

Iron corrected T1 is an imaging biomarker that characterises tissue by the proportion of the extracellular fluid present within the liver tissue. Iron corrected T1 corrects for the effects of elevated iron on the T1 measured signal, and can thus be thought of as the T1 value of liver tissue that would be measured at a normal hepatic iron level of 1.3mg/g. Iron corrected T1 values in the liver have been shown to correlate with both inflammation and fibrosis histology [2].

See Volume 002, Document 001: Iron Corrected T1 Biomarker Description – RA251 for a more complete description of the proposed biomarker.

E. Measurement and Regulated Diagnostics

Iron corrected T1 is an imaging biomarker, using MR as imaging modality. Iron corrected T1 is computed as per the published algorithm [1] and is quantified using post-processing software. There are several regulated diagnostics cleared under the 510(k) pathway which can compute cT1:

- K172685, manufactured by Perspectum Diagnostics Ltd
- K143020, manufactured by Mirada Medical Ltd

F. Limitations of Use

The cT1 biomarker is not appropriate for potential participants where MR is contra-indicated.

G. Evaluating Efficacy/Safety

The cT1 biomarker does not introduce uncertainty when evaluating efficacy or safety. The proposed COU calls for a biopsy to confirm the enrollment criteria had been met. cT1 is only proposed as a diagnostic enrichment biomarker for use during screening, to rule out patients unlikely to meet histopathological enrollment criteria.

H. Application to Multiple Drug Development Programs

The proposed COU limits the use of the biomarker to drug development programs focussed on NASH.

III. Biomarker Information

A. Name, Source, Type, Description

Iron corrected T1 is an imaging biomarker, derived from MR images using 510(k) cleared post-processing software. A 15 minute (including patient setup) MR examination is required to capture MR data. The mid-abdominal MR examination includes two short breath hold sequences and does not require the use of contrasting agent. MR data is fed into a 510(k) cleared regulated diagnostic product to produce the quantitative cT1 biomarker.

B. Interpretation

Iron corrected T1 is a biomarker that quantifies the T1 MR relaxation time of liver tissue and corrects for the local magnetic effects exerted by hepatic iron that artificially shorten the T1 measurement. Corrected T1 relates to the amount of extracellular fluid present in liver parenchyma. cT1 is measured in milliseconds (unit: ms). Corrected T1 values in the liver have been shown to correlate with both disease activity (inflammation and ballooning) and fibrosis histology [2].

C. Rationale

In order to understand the proposed biomarker, iron corrected T1 (cT1), we first need to introduce T2* and T1.

T2*

In general, T2* is a measure of the transverse (spin-spin) relaxation time of a substance. The T2* of a tissue is affected by local magnetic susceptibility effects, including those caused by iron deposits. In the liver, iron deposits, typically in the form of ferritin and hemosiderin, cause inhomogeneities in the magnetic field that have a measurable effect on T2*. T2* is measured in milliseconds (unit: ms) and is field strength dependent.

It follows that T2* is a metric that relates to iron content of the liver tissue, at a specific field strength. Tissues with high iron typically have very short values of T2*, while tissues with very low iron have longer values. The relationship between T2* and liver iron concentration (unit: mg Fe/g dry weight tissue) has been formally characterised [29].

T1

In general, T1 is a measure of the longitudinal (spin-lattice) relaxation time of a substance. The T1 of a tissue is influenced by the free water content, which relates to the proportion of the extracellular fluid in the tissue. It is also affected by local iron deposits, which act to reduce T1. T1 is measured in milliseconds (unit: ms) and is field strength dependent.

Proton-dense tissues with a low water content, such as fat, have very short T1s, while tissues with a higher water content, such as muscle and the spleen, have much longer T1s. When tissue is inflamed or scarred (fibrotic), changes in the structural organization of the tissue, due to tissue remodelling, mean that the water content increases, leading to longer T1 values.

The MRI metric T1 is increasingly used in the field of cardiology to assess heart health, acting as a surrogate for inflammation and fibrosis in the myocardium (the heart ventricle muscle wall) [30]. This has been aided by the development of T1 mapping software by the main MRI manufacturers, such as MyoMaps from Siemens (US FDA 510(k) K141977), CardiacQuant from Philips (US FDA 510(k) K153324) and CardioMaps from GE (US FDA 510(k) K163331). The application of T1 as a biomarker of activity (inflammation and ballooning) and fibrosis in the liver is impeded by the high prevalence of elevated liver iron [25, 31].

Inflammation, ballooning and fibrosis are both pathophysiological processes in many forms of liver disease. However, unlike in heart disease, patients with liver disorders are significantly more likely to have iron overload [23, 25-28], which causes T1 measurements to decrease. If this is not taken into account, there is a risk of underestimation of disease: in a conventional T1 map, the local magnetic effects exerted by the iron artificially shorten the T1 measurement, leading to potential underestimation of disease.

Iron Corrected T1

It is possible to use the T2* map to correct for signal changes related to iron deposits, producing a corrected T1 map, referred to as cT1. This iron-corrected T1 map eliminates the effects of elevated iron from T1 measurements [2]. Refer to Figure 2 for a diagrammatic overview.

Corrected T1 relates to the amount of extracellular fluid present in liver parenchyma.

Like T2* and T1, cT1 is measured in milliseconds (unit: ms). Furthermore, cT1 is standardised across MR manufacturers and normalised to a single field strength[3]. It follows that cT1 is field strength independent.

Iron corrected T1 can therefore be thought of as the T1 value that would be measured on a 3T reference scanner at a normal hepatic iron level of 1.3mg/g. That is, by using T2* we can correct for iron and produce an iron corrected T1 measurement. Corrected T1 values in the liver have been shown to correlate with both inflammation and fibrosis histology [2].

[3] cT1 is standardised across different MRI vendors, and normalised to the T1 corrected for iron as reported on a 3T reference scanner (Siemens Skyra VE11 with MyoMaps) using a Modified Look Locker Inversion (MOLLI) Recovery Sequence.

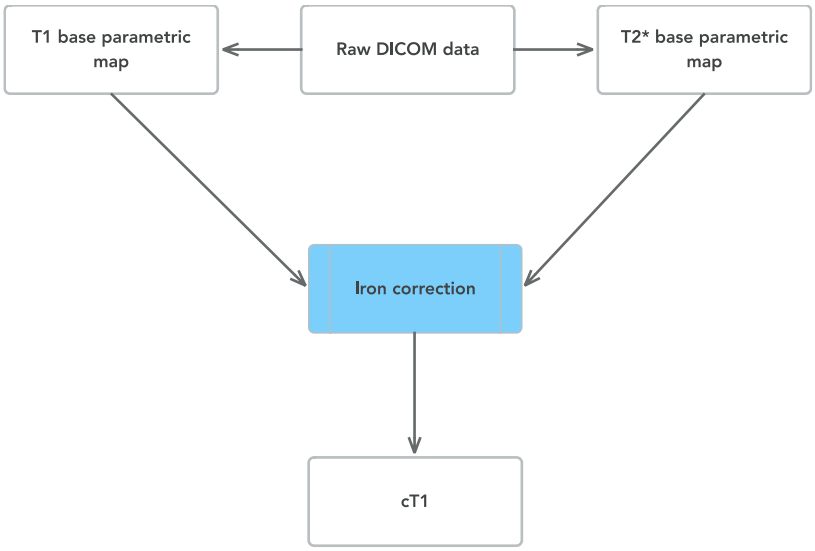

Figure 2 – Inputs to computing cT1.

IV. Biomarker Measurement Information

A. General Description of Biomarker Measurement

A high level overview of the measurement methodology can be summarised thus.

- An MR radiographer acquires MR data as per the acquisition protocols supported by LiverMultiScan.
- The raw MRI data is fed into LiverMultiScan.
- The data is analysed by an operator as per the workflow of the LiverMultiScan device.
- A pdf report is produced, containing the cT1 biomarker.

Most biomarkers will be measured using a device that performs the actual measuring procedure. We establish essential methodological requirements that need to be satisfied for cT1 to be recognised as a biomarker, and provide reference to the evidence to support qualification.

We present an overview of the measurement methodology: the acquisition protocols used to acquire MRI data, as well as a description of two post-processing, commercially available devices (K143020 and K172685) used to quantify cT1 data from raw MRI data.

Requirement	To Demonstrate	Reference / Justification
Multiple devices that reliably and accurately measure a qualified biomarker are expected to yield the same results	A comparison between K143020 and K172685	Iron Corrected T1 Device Comparison Performance Testing – RA257
The analytical performance of the device will be considered	Accuracy and precision of K172685 in terms of	Iron Corrected T1 Bench Performance Testing – RA258

during the evaluation of the biomarker for qualification	repeatability and reproducibility	Iron Corrected T1 Clinical Performance Testing – RA259
The biomarker needs to be conceptually independent from the measurement device	cT1 is conceptually independent from the devices used to measure it	Corrected T1 is presented as an independent concept. Reference is made to two measuring devices, manufactured by two different manufacturers. The use of input data, acquired on different MR manufacturers, is presented
The biomarker cT1 should be standardised to deal with MR manufacturer differences, different scanner models and field strengths (1.5 T and 3T)	Iron Corrected T1 is normalised to 3T (regardless of the field strength of the measuring MR system) and standardised across manufacturers and field strengths	Iron Corrected T1 Normalisation and Standardisation – RA260

B. Test/Assay Information

Indicate whether the biomarker test/assay is one or more of the following:

i. Laboratory Developed Test (LDT) No
ii. Research Use Only (RUO) No
iii. FDA Cleared/Approved Yes
If yes, provide 510(k)/PMA #: K172685
iv. If the biomarker is qualified, will the test/assay be performed in a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory? No
v. Is the biomarker test currently under review by the Center for Devices and Radiological Health or the Center for Biologics Evaluation and Research? No
vi. Is there a standard operating procedure (SOP) for sample collection and storage? No
vii. Is there a laboratory SOP for the test/assay methodology? No

C. Biomarker Measurement

Quality Assurance

The quantification of corrected T1 is contingent on the acquisition of data of sufficient quality. MR Radiographers / technicians are provided with training and acquisition manuals to ensure robust acquisitions.

However, once received, all data goes through automated Quality Control (QC) checks. Current automated checks (by US FDA 510(k) cleared device K172685) include:

- Checking inversion times to ensure the data is acquired as per our acquisition protocol
- Ensuring correct shim box positioning

- Automated checks to ensure data was acquired with a supported scanner

Once a dataset passes the automated QC checks, the operator of the device may identify potential problems:

- Incorrect anatomical position, by identifying the liver and the surrounding anatomy
- Unacceptable signal to noise (SNR) ratio
- Abrupt change in intensity/colour, or an inhomogeneous texture that cannot be explained by anatomy
- T1 and T2* are not within the range of reasonably possible values, both within and outside the liver
- Left/right disparity in values in the liver
- Image artefacts:
    - evidence of motion (i.e. repeated motifs)
    - evidence of shimming artefact (abrupt drop in signal)
    - field heterogeneity (gradient across slice)
    - mistriggering (difference in values between slices)
    - low SNR (noise around and within abdomen)
    - presence of contrast agent
    - scanned off isocentre
    - fat suppression turned on

A list of cautions and informational messages, within the context of K172685, is provided.

Type	Message
	No valid DICOM data was found in the specified directory.
	Caution: the folder you have selected contains multiple DICOM studies.
	Caution: the DICOM Study you have selected cannot be used for analysis.
	Caution: The selected T1 Series show evidence of atypical RR intervals. This may generate misleading results.
	One or more of the selected T2* Series does not contain enough in-phase images for processing.
	Caution: One or more of the selected T1 Series show evidence of the wrong number of epochs.
	Caution: You have selected one or more Series that have been acquired with slices outside the shim box. This may generate misleading results.

	No T1 data is available for loading. If you continue, the system will only generate T2* and PDFF images.
	Caution: cT1 ROI [x] show an excessive degree of variation (IQR difference greater than 100ms). This may result in inaccurate results.
	Caution: cT1 slice(s) 1 and 2 shows evidence of an atypical RR interval. This may generate misleading results.

Limitations on Image Acquisition

The cT1 metric is derived from T1 and T2* maps which are generated respectively from a Modified Look Locker Inversion (MOLLI) recovery sequence and a multi-echo spoiled-gradient acquisition. MR radiographers and/or technicians are provided with sequence-specific training as well as acquisition manuals. Anything that can compromise the acquisitions, can compromise cT1.

Patient preparation is critical prior to acquisition: the patient must be in the correct position on the MR scanning table; body coil arrays must be positioned correctly on top of the abdomen; the RF-shielded magnet room door should be closed; and the acquisition slice must be positioned close to magnet isocentre to minimize signal inhomogeneities. These are all standard data acquisition practices. The principal source of artefacts in abdominal MRI imaging is from breathing or movement. The acquisitions are done at end-expiration breath-hold, but sometimes the patient cannot hold their breath for the full scan time (8-10sec depending on heart rate). As a result, motion artefacts can occur, which is most common with the T2* acquisition. Cardiac gating is used and this needs setting up on the patient. Heart rate mistriggering can also cause under- or overestimations of T1 values, but the MR radiographer can monitor[4] mistriggering (and, if necessary, reacquire the data). This can be detected during post-processing, at which point erroneous data can only be discarded.

Poor shimming (which means poor magnetic field homogeneity) can cause variations in T1 values across the liver and cause the T2* signal to decay too quickly irrespective of iron overload. Poor shimming can also cause what are known as banding artefacts in the balanced steady state free precession readout of MOLLI sequence (signal drop outs in the image caused by frequency offsets). For this reason, the shim volume must be placed so that it covers the part of the liver that is imaged. MR radiographers are trained to place shim volumes appropriately, and our acquisition manuals have numerous explanatory pictures to aid with this task.

Other sources of error come from MR technologists changing protocol parameters, which could cause changes in the estimated relaxation times and impair cT1 standardization across scanners. If

[4] As described in the acquisition materials and highlighted during training.

this does occur, feedback is provided to the MR technologists upon data analysis when the data is received after the scan to ensure the protocol parameters are corrected.

Other limitations include the standard contraindications to MRI scanning, that can include pacemakers, certain metallic implants, claustrophobia etc.

D. Additional Considerations for Radiographic Biomarkers

Measurement information related to cT1 can be found in Volume 003 and Volume 004.

- Volume 003, Document 001: Measuring Iron Corrected T1 – RA252:
  - A general overview of the process used to measure cT1
  - A description of the MRI acquisition protocols
  - A description of the 510(k) cleared, post- processing software device, LiverMultiScan, used to measure the biomarker
  - Device performance characteristics summary
- Volume 003, Document 002: Iron Corrected T1 Device Comparison Performance Testing – RA257:
  - Performance evaluation to demonstrate that the cT1 biomarker, as measured by two devices commercially available and cleared by the FDA, yields equivalent results
- Volume 003, Document 003: Iron Corrected T1 Bench Performance Testing – RA258:
  - Performance evaluation using phantoms to demonstrate accuracy of cT1
  - Performance evaluation using phantoms to demonstrate repeatability of cT1
  - Performance evaluation using phantoms to demonstrate reproducibility of cT1
- Volume 003, Document 004: Iron Corrected T1 Clinical Performance Testing – RA259:
  - Performance evaluation using volunteers across a range of cT1 values to demonstrate repeatability of cT1
  - Performance evaluation using volunteers across a range of cT1 values to demonstrate reproducibility of cT1
- Volume 004, Document 001: Iron Corrected T1 Operator Reliability Assessment – RA250
  - Reliability assessment of the operators of the LiverMultiScan device in placing Regions Of Interest (ROIs)

VIII. Assessment of Benefits and Risks

Benefits	Individual potential participants may benefit from the use of the cT1 biomarker by avoiding the need for unnecessary biopsies in those participants who are unlikely to meet the histopathological enrollment criteria. Participants will only undergo a confirmatory biopsy if the cT1 cut-off threshold of 800ms is met.

Risks	The use of the cT1 as a diagnostic enrichment biomarker during screening may potentially miss participants who would have ordinarily met histopathological enrollment criteria (false negatives).
Risk Mitigations	The cT1 cut-off of 800ms has been selected in the test dataset and confirmed in the validation dataset to optimise sensitivity.
Conclusion	Benefits outweigh any potential risks. Adequate risk mitigations reduce risk as far as reasonably practicable. A proportion of the potential participants avoid the need for a risky and unnecessary biopsy.

Benefits	Individual potential participants may benefit from the use of the cT1 biomarker by avoiding the need for unnecessary biopsies in those participants who are unlikely to meet the histopathological enrollment criteria. Participants will only undergo a confirmatory biopsy if the cT1 cut-off threshold of 800ms is met.
Risks	The use of the cT1 as a diagnostic enrichment biomarker during screening may potentially recommend a confirmatory biopsy in participants who do not meet the histopathological enrollment criteria (false positives).
Risk Mitigations	Selection of 800ms as a cT1 cut-off optimises the diagnostic sensitivity, thus reducing the number of unnecessary biopsies. Independent test and validation datasets are used.
Conclusion	Benefits outweigh any potential risks. Adequate risk mitigations reduces risk as far as reasonably practicable. Under a conventional screening regime (where the cT1 biomarker is not used within the proposed COU) the potential participants would have undergone the biopsy in all eventualities. The use of the cT1 biomarker within this COU does not expose additional participants to biopsy.

Benefits	The use of cT1 as a diagnostic enrichment biomarker for use during screening may facilitate better enrollment in NASH trials, as participants are more likely to agree to a confirmatory biopsy due to increased likelihood of histopathological findings that meet enrollment criteria, compared to a conventional approach where no non-invasive pre-screening steps are taken.
Risks	The use of the cT1 as a diagnostic enrichment biomarker may systematically exclude certain participant demographics.
Risk Mitigations	The use of cT1 within the proposed COU was validated in independent training and validation cohorts. Available baseline demographic data for both cohorts suggests that there are no systematic exclusions when cT1 is used within the proposed COU.
Conclusion	Benefits outweigh any potential risks. Adequate risk mitigations reduce risk as far as reasonably practicable. A proportion of the potential participants avoid the need for a risky and unnecessary biopsy.

The use of non-invasive diagnostics to recommend potential participants for confirmatory biopsy would facilitate better enrollment in NASH trials, as participants are more likely to agree to a biopsy if all reasonable steps had been taken to increase the chances of a successful, mandated biopsy. The use of

cT1 does not preclude the use of any existing diagnostics currently used, nor does it replace the need for a confirmatory biopsy. The benefits of reducing potential unnecessary biopsies outweigh any risks.

IX. Evaluation of Existing Biomarker Information: Summaries

A. Pre-Clinical Information, as appropriate

None for the proposed COU.

B. Completed Clinical Information, as appropriate

The rationale for the cT1 biomarker and support for its use within the proposed COU is demonstrated in two independent cohorts:

- The training cohort, a UK-based population in which the proposed cT1 cut-off of 800ms is systematically derived
- The validation cohort, a US-based population in which the use of the proposed cT1 cut-off is demonstrated

Training Cohort

Iron corrected T1 (cT1) metrics were quantified from raw MR data acquired on a sample of n=102 biopsy confirmed NAFLD participants. The training dataset was pooled from two similar UK-based cohorts into participants with liver disease. Full study protocols are included with this submission.

- RIAL-NICOLA (n=53). See RIAL-NICOLA Protocol – RA253
- CALM (n=49). See CALM Protocol – RA254

Criteria	RIAL-NICOLA	CALM
Description	Male and female adult participants due to undergo liver biopsy to establish a diagnosis of liver disease as part of standard of care. Patients to undergo an MRI examination and transient elastography. These will be compared to the findings from the liver biopsy to determine the degree of correlation	Male and female adult participants booked for non-targeted liver biopsy for any indication were prospectively recruited to undergo MRI examination. The results from the MRI examination will be compared to the findings from the liver biopsy to validate corrected T1
Inclusion criteria	Participant is willing and able to give informed consent for participation in the study Male or female over 18 years due for diagnostic liver biopsy	Patients with a histologically confirmed diagnosis of NAFLD without secondary cause and without history of alcohol excess

Criteria	RIAL-NICOLA	CALM
Exclusion criteria	• Contraindications to MRI scanning • Consumed more alcohol than the current limit recommended by the UK Department of Health: 3-4 units [24-32 g]/day for males and 2-3 units [16-24 g]/day for females • Had clinical or laboratory evidence of a liver diagnosis other than NAFLD	• Any contraindication to MRI • Alcohol consumption of > 21 UK units/week for males and >14 UK units/week for females • Biopsy of a distinct focal lesion • Inability to give fully informed consent

Validation Cohort

In order to validate the established cT1 cut-off of 800ms, this threshold is applied to an independent validation dataset. This sample was taken from a US cohort of n=135 biopsy-confirmed NAFLD patients from the BAMC prevalence study. Participants were recruited as part of a study into prevalence of liver disease in those referred for routine colorectal cancer screening with no prior history of liver disease or alcohol abuse. MRI data was acquired as part of the screening protocol prior to staging for liver biopsy. As a consequence of the nature of recruitment being patients suspected of having liver disease rather than those with biopsy-confirmed liver disease, the proportion of patients with NAS≥4 and F≥2 was 12% lower than in the training dataset. There were also differences in the ethnicity of the groups, with 39% of this sample from Hispanic descent, whereas 90% of the individuals in the training sample were from white British descent. Despite these differences, an enrichment of 82% was achieved at the proposed cT1 cut-off threshold of 800ms for distinguishing NAS≥4 and F2-F3.

Male and female adults (between 18 to 80 years of age) who meet the qualifying criteria for percutaneous liver biopsy were recruited to participate in the study. Participants were ineligible based on the following exclusion criteria:

- Patients with excessive alcohol use as defined as >21 units of alcohol/week for men and 14 units of alcohol/week for women over a 2-year time frame. One drink unit or one standard drink is equivalent to a 12-ounce beer, a 4-ounce glass of wine, or a 1-ounce shot of hard liquor
- Patients with prior history of liver disease including chronic hepatitis B or C, hemochromatosis, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, HIV, or prior documentation of NAFLD
- Patients on medications known to cause fatty liver disease: tamoxifen, corticosteroids, amiodarone, methotrexate, valproic acid
- Patients carrying an implantable active medical device such as a pacemaker or a defibrillator
- Pregnant women

Training and Validation Results

Refer to Volume 005 for information on the training cohort and how the proposed cT1 cut-off is derived:

- Volume 005, Document 001: Iron Corrected T1 Training Report – RA249
- Volume 005, Document 002: RIAL-NICOLA Protocol – RA253
- Volume 005, Document 003: CALM Protocol – RA254

Refer to Volume 006 for information on the validation cohort and a demonstration of the utility of cT1 within the proposed COU:

- Volume 006, Document 001: Iron Corrected T1 Validation Report – RA248
- Volume 006, Document 002: PREV Protocol – RA255

These volumes contain study protocols, baseline demographics, histopathological results, serological as well as imaging biomarkers (cT1, MR Elastography and/or Transient Elastography).

C. Summary of Ongoing Information Collection/Analysis Efforts

None for the proposed COU.

X. Knowledge Gaps in Biomarker Development

A. List and describe any knowledge gaps, including any assumptions, that exist in the application of the biomarker for the proposed COU

None for the proposed COU.

B. List and describe the approach/tools you propose to use to fill in the above-named gaps when evidence is unknown or uncertain, (i.e., statistical measures and models, meta-analysis from other clinical trials).

None for the proposed COU.

C. Describe the status of other work currently underway and planned for the future toward qualification of this biomarker for the proposed context of use.

None for the proposed COU.

Attachments

Reference Number	Reference Description
RA152	Cover Letter – RA152
RA153	LOI Feedback – RA153
RA154	Table of Contents – RA154
RA172	Premeeting Comments – RA172
RA216	Summary of Face-to-Face Meeting Minutes – RA216
RA217	BQRT Questions and Sponsor Responses – RA217
RA246	Sponsor Questions – RA246
RA247	Iron Corrected T1 Biomarker Qualification Plan – RA247
RA248	Iron Corrected T1 Validation Report – RA248
RA249	Iron Corrected T1 Training Report – RA249
RA250	Iron Corrected T1 Operator Reliability Assessment – RA250
RA251	Iron Corrected T1 Biomarker Description – RA251
RA252	Measuring Iron Corrected T1 – RA252
RA253	RIAL-NICOLA Protocol – RA253
RA254	CALM Protocol – RA254
RA255	PREV Protocol – RA255
RA257	Iron Corrected T1 Device Comparison Performance Testing – RA257
RA258	Iron Corrected T1 Bench Performance Testing – RA258
RA259	Iron Corrected T1 Clinical Performance Testing – RA259
RA260	Iron Corrected T1 Normalisation and Standardisation – RA260
RA409	Iron Corrected T1 Legacy Biomarker Status Update Summary – RA409
RA410	Chris Leptak Email – RA410

Vol	Doc	Pages	Topic
Root	001	2	Cover Letter – RA152
Root	002	7	Table of Contents – RA154Table of Contents – RA154
001			Legacy Biomarker Status Update Summary
001	001	24	Iron Corrected T1 Legacy Biomarker Status Update Summary – RA409
002			Biomarker Description
002	001	14	Iron Corrected T1 Biomarker Description – RA251
003			Measuring Iron Corrected T1
003	001	14	Measuring Iron Corrected T1 – RA252
003	002	8	Iron Corrected T1 Device Comparison Performance Testing – RA257
003	003	35	Iron Corrected T1 Bench Performance Testing – RA258
003	004	18	Iron Corrected T1 Clinical Performance Testing – RA259
003	005	16	Iron Corrected T1 Normalisation and Standardisation – RA260
004			Operator Reliability
004	001	10	Iron Corrected T1 Operator Reliability Assessment – RA250
005			Training Data
005	001	25	Iron Corrected T1 Training Report – RA249
005	002	16	RIAL-NICOLA Protocol – RA253

005	003	14	CALM Protocol – RA254
006			Validation Data
006	001	19	Iron Corrected T1 Validation Report – RA248
006	002	16	PREV Protocol – RA255
007			Biomarker Qualification Plan
007	001	7	Iron Corrected T1 Biomarker Qualification Plan – RA247
008			BQRT Questions
008	001	24	BQRT Questions and Sponsor Responses – RA217
009			Sponsor Questions
009	001	6	Sponsor Questions – RA246
010			Previous Discussions or Submissions
010	001	4	LOI Feedback – RA153
010	002	4	Premeeting Comments – RA172
010	003	6	Summary of Face-to-Face Meeting Minutes – RA216
010	004	2	Chris Leptak Email – RA410
011			Referenced Literature
011	001	9	Alam 2016 – J Cardiovasc Magn Reson
011	002	7	Anastasiou 2010 – Eur J Gastroenterol Hepatol
011	003	9	Banerjee 2014 – J Hepatol
011	004	5	Bedossa 1996 – Hepatology
011	005	9	Bedossa 2012 – Hepatology
011	006	11	Bedossa 2014 – Hepatology
011	007	9	Bland 1986 – Lancet
011	008	3	Bozzini 2005 – Diabetes Care
011	009	8	Brunt 1999 – Am J Gastroeneterol
011	010	9	Bugianesi 2004 – Hepatology
011	011	5	Cadranel 2000 – Hepatology
011	012	12	Dabir 2014 – J Cardiovasc Magn reson
011	013	6	Dixon 1981 – Radiology
011	014	13	Dongiovanni 2011 – J Hepatol
011	015	18	Ghugre 2005 – Magn Reson Med
011	016	1	Ghugre 2008 – Proc Intl Soc Magn
011	017	13	Glover 1991 – Magn Reson Med
011	018	6	Goldin 1996 – J Hepatol
011	019	9	Henninger 2012 – Eur Radiol
011	020	9	Hoad 2015 – NMR Biomed
011	021	4	Ishak 1995 – J Hepatol
011	022	7	Jehn 2004 – Diabetes Care
011	023	20	Kellman 2014 – J Cardiovasc Magn Reson
011	024	9	Kleiner 2005 – Heaptology
011	025	5	Knodell 1981 – Hepatology
011	026	6	Mamisch 2012 – Skeletal Radiol
011	027	12	Moon 2013 – J Cardiov Magn Reson
011	028	16	Nacif 2001 – J Magn Reson Imaging
011	029	6	Nelder 1965 – Comput J
011	030	9	Nelson 2012 – Curr Gastroenterol Rep

011	031	7	Oseini 2017 – Liver Int
011	032	11	Piechnik 2010 – J Cardiov Magn Reson
011	033	2	Piechnik 2015 – J Cardiovasc Magn REson
011	034	2	Rohrer 2005 – Invest Radiol
011	035	19	Sanyal 2011 – Hepatology
011	036	3	Scheuer 1991 – J Hepatol
011	037	4	Sherman 2015 – Hepatology
011	038	7	St Pierre 2005 – Blood
011	039	15	Taylor 2016 – JACC Cardiovascular Imaging
011	040	5	Trotter 2006 – Clin Liver Dis
011	041	13	Tunnicliffe 2016 – J Magn Reson Imaging
011	042	17	Unal 2017 – Expert Rev Gastroenterol Hepatol
011	043	7	Valenti 2003 – Dig Liver Dis
011	044	8	Valenti 2010 – Gastroenterol
011	045	7	Valenti 2010 – J Hepatol
011	046	5	Valenti 2012 – World J Gastroenterol
011	047	4	Varma 2014 – Intern Med J
011	048	6	Vymazal 1996 – Magn Reson Med
011	049	7	Wood 2005 - Blood

APPENDIX I.

Publications/References

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