# Quantifying the perturbation of gadoxetate kinetics by metformin and ciclosporin with functional magnetic resonance imaging of the liver.

Study protocol

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# Research Protocol

**Full Title:** Quantifying the perturbation of gadoxetate kinetics by metformin and ciclosporin with functional magnetic resonance imaging of the liver.

**Short Title:** Assessing changes in gadoxetate metabolism by the liver using MRI

**Study Sponsor:** Sheffield Teaching Hospitals NHS Foundation Trust

**Investigators:**

- Sheffield Teaching Hospitals
    - (Clinical Lead and principal investigator) Benjamin Rea (Consultant GI radiologist and honorary senior lecturer)
- University of Sheffield
    - (University Lead) Steven Sourbron (Professor of imaging physics)
- Bioxydyn Ltd
    - Geoff Parker (Founder)
    - John Waterton (Translational Imaging Specialist)

**Estimated Duration of Study:**

- 3 months
    - Start Date: 30.9.24
    - End Date: 28.02.25

**Number and Type of Participants:**

- Enrolment
    - 12 healthy volunteer participants (6 for each of metformin and ciclosporin groups)
- Sex
    - Equal number of males and females for each drug
- Age Range
    - ≥ 18 years

# Synopsis

## Background

The identification of drugs at risk of drug-drug interactions (DDI) early in the drug development life cycle is key to avoid late stage drug development failures. A critical gap in current methodologies is for tools that are widely available and safe to use in humans, and specifically can distinguish between perturbation of hepatocellular uptake, excretion or both.

Dynamic gadoxetate enhanced magnetic resonance imaging (DGE-MRI) is a technique that can potentially fill this gap. The MRI contrast agent gadoxetate is used in clinical routine, it is known to be taken up in hepatocytes by transporters OATPB1 and excreted to bile by MRP2 transporters, and the respective uptake and excretion rates can be quantified with DGE-MRI using suitable MRI scans and data modelling.

Studies in animal models using DGE-MRI on 6 different drugs have clearly demonstrated various levels of drug-induced inhibition of gadoxetate uptake and excretion (Karageorgis, 2018; Melillo, 2023). Recently, a proof-of-concept study in healthy human volunteers using DGE-MRI to characterise a single drug (rifampicin) has shown a systematic 95% reduction in gadoxetate uptake and 40% reduction in excretion.

## Aims

### Study objectives

The purpose of the current study is to expand on these previous results and use DGE-MRI to measure the inhibition of gadoxetate uptake and excretion in volunteers with two other test drugs, metformin and ciclosporin (Neoral). These drugs are selected because they are commonly used in clinical practice, have a good safety profile and are known to inhibit OATP1B1 and MRP2 function (Ruan, 2023; Gertz, 2013). They should therefore also produce a measurable effect on gadoxetate uptake and excretion rates. If we can show that this is indeed

measurable, the results will add further evidence that these effects can be detected by DGE-MRI, improve our understanding of relevant effect size and limits of detection, and help us identify thresholds above which reduction in gadoxetate uptake or excretion would be of concern.

### Study endpoints

1. The effect of a clinical dose of metformin on hepatocellular gadoxetate uptake and excretion in healthy volunteers.

2. The effect of a clinical dose of ciclosporin (Neoral) on hepatocellular gadoxetate uptake and excretion in healthy volunteers.

### Long-term aims

On the longer term we expect these data to help build a case that DGE-MRI can be a useful tool to assess transported-mediated DDI risk in early drug development. The study results will be included in an application to FDA's biomarker qualification program as described in a biomarker qualification plan (submitted to FDA) following acceptance by the FDA of a letter of intent outlining the rationale for this biomarker in drug development.

## Methods

This is a single centre, prospective observational study including healthy volunteer participants. 6 participants will be recruited to each immediate-release metformin and ciclosporin (Neoral) drug arms (12 total). After screening and consent, each subject will attend two hospital visits. On each visit they will undergo DGE-MRI in the morning, and once again after a 2-hour break to allow for sufficient time to measure biliary gadoxetate clearance. On the second visit one of the two test drugs will be administered before the first scan. Each DGE-MRI scan will be performed using 25% (¼) of a clinical dose of gadoxetate. Immediate-release metformin and ciclosporin (Neoral) will be used at standardised dosages (1000mg and 100mg, respectively). Blood samples will be taken before drug administration and before each scan for comparative assessment with liver function tests (LFT).

A schema of this work is outlined below and in Figure 1:

1. **Visit A, Screening:** After eligibility screening, informed consent for participation will be taken. A clinical examination including observations and blood tests for renal and liver function will be taken. If renal function shows eGFR<30mL/min/1.73m2, liver function parameters exceed laboratory normal values or clinical examination findings are not compatible with the inclusion criteria, the participants will be removed from the study.
2. **Visit B, Baseline Scan:** Participants will have two DGE-MRI scans with 25% (¼) standard clinical dose of gadoxetate administered at each. Blood samples (<5mL) will be taken before each scan to assess liver function.
3. **Visit C, Treatment Scan**: After giving informed consent, participants will receive a single dose of either metformin or ciclosporin (Neoral) followed by an initial DGE-MRI scan at 25% (¼) standard clinical dose of gadoxetate. Metformin (1000mg) or ciclosporin (100mg, Neoral) will be administered 2 and 1 hour before gadoxetate injection, respectively. This is followed by another DGE-MRI at the same dose after a break of 60-120 minutes. LFTs (<5mL blood) will be taken before metformin/ciclosporin (Neoral) administration, and also before each scan (totalling 3 blood tests).

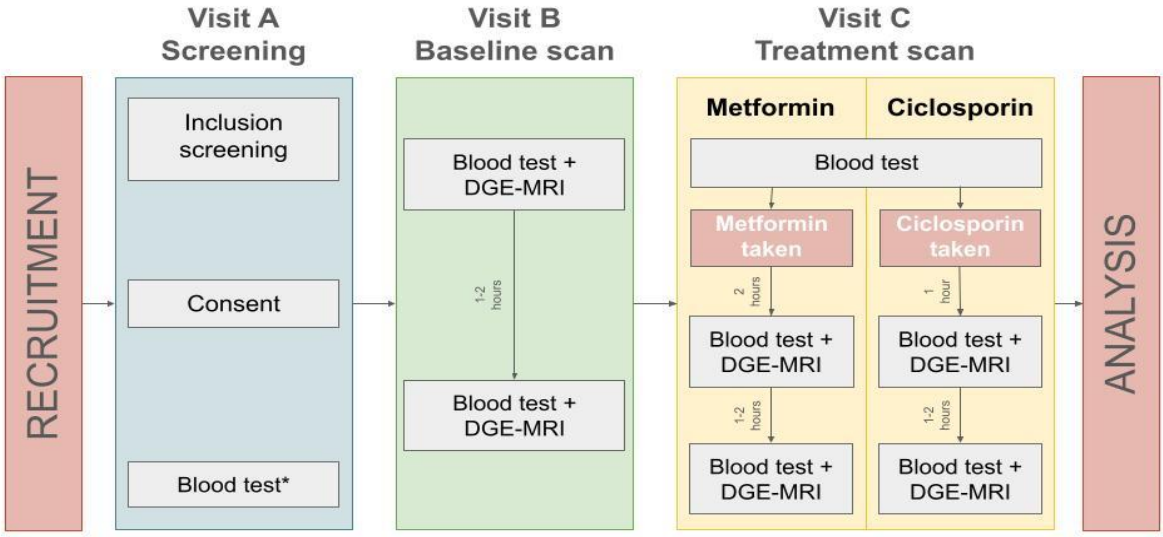


**Figure 1.** *Study flow diagram.*

## Analyses

All images will be reviewed by a qualified abdominal radiologist to check for incidental findings.

Gadoxetate uptake and excretion kinetic rate constants will be measured with and without the drug for all participants. The primary outcome measure is the effect size of the drug on uptake and excretion.

The primary analyses will be done at the University of Sheffield.

## Safety

The modelling and use of gadoxetate as a research biomarker was established in the proof-of-concept study conducted at the University of Leeds. Gadoxetate will be used at 25% (¼) standard clinical dose for each scan. This dose was established in the precursor study as the requisite dose to enable analyses of gadoxetate kinetics whilst not exposing participants unnecessarily to higher doses of the gadoxetate. At 25% of standard clinical dose, gadoxetate is not licensed for the diagnostic use. The administration of gadoxetate will be authorised by the clinical lead, Dr Benjamin Rea.

Both Metformin and Ciclosporin have an excellent safety profile and record, and therefore the risk posed to a healthy volunteer with no history of polypharmacy or comorbidities from a one off dose, is negligible. Routine clinical exclusions will be applied pertaining to the administration of gadoxetate, metformin and ciclosporin (Neoral) as well as contraindications to MRI imaging.

There will be no monitoring procedures undertaken following completion of this study but the research fellow will remain contactable following completion of the study for further queries.

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# 1. Background

## 1.1 The TRISTAN project

This study is part of an international project TRISTAN (Translational Imaging in Drug Safety Assessment), which receives funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 116106. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and the European Federation of Pharmaceutical Industries and Associations (EFPIA). The primary aim of TRISTAN is to develop and validate imaging biomarkers for drug safety assessment.

Currently, imaging biomarkers are not widely used in the drug discovery process although they could advance drug safety evaluation, both for pre-clinical and clinical development. They have the potential to improve translatability of preclinical (animal) data to healthy volunteers and patients and thus could help avoid late stage attrition of development programmes. In addition, functional diagnostic imaging methods used as biomarkers would offer the possibility to confirm drug toxicity mechanisms in humans, including the potential to determine drug-drug interactions (DDI's).

TRISTAN (www.imi-tristan.eu) is a public-private partnership involving 21 organisations including academic centres, research organisations, small and medium-size enterprises (SMEs), imaging and pharmaceutical companies which form the Consortium under the consortium agreement. The collective objective is to validate or qualify translational imaging methods as potential imaging biomarkers, specifically addressing three areas with a high unmet medical need - the assessment of liver toxicity, lung toxicity and the bio-distribution of biologics.

The project is organised in five work packages - WP1 orchestrates management, communication, dissemination and ethics; WP2 aims to develop a DGE-MRI biomarker assay for early identification of possible drug-drug interactions (DDIs);

WP3 aims to develop imaging biomarkers for drug-induced interstitial lung disease (DIILD); WP4 aims to characterise the bio-distribution of biologics; and WP5 will define a roadmap for dissemination of the imaging biomarker assays. The current study is organised by TRISTAN WP2.

## 1.2 Motivation

Many hundreds of licensed drugs may cause undesired interactions with co-administered victim drugs (DDIs) or drug induced liver injury (DILI). DDIs can result in potentiated or reduced efficacy that requires drug dose adjustment, and/or increased or reduced toxicity to liver or other tissues, for example muscle toxicity caused by statins (Guo et al., 2018). DILI may result in severe symptomatic liver injury and/or life-threatening acute liver failure (Andrade et al., 2019) and is a consequence of adverse effects of drugs on liver function, plus susceptibility factors that in general are poorly understood. For all but a few drugs the frequency of human DILI is low liver injury is not evident in animal safety studies. This is likely to be because the susceptibility factors arise only infrequently and unpredictably (Andrade et al., 2019). Consequently, DILI typically is not recognised prior to Phase 3 clinical trials, or post-marketing.

One important mechanism that can initiate both DDIs and DILI is perturbation by drugs of the activities of membrane transport proteins on hepatocytes. For example, inhibition of the hepatic uptake transporter OATP1B1 by a co-administered perpetrator drug may impair clearance of statins and lead to elevated plasma and systemic tissue exposure, resulting in myotoxicity (Guo et al., 2018). Conversely, interaction of perpetrator drugs with hepatic transporters that mediate biliary excretion may alter hepatocyte exposure to a victim drug without causing a measurable effect on systemic plasma exposure (e.g., metformin DDIs due to OCT2/MATE inhibition) (Galetin et al., 2017). The hepatocyte transporter interaction proposed to play a role in DILI initiation is the Bile Salt Export Pump (BSEP). BSEP mediates excretion of bile salts from hepatocytes into bile and genetically inherited defects in human BSEP expression are known to cause severe cholestatic liver injury, which arises because elevated bile salt concentrations within hepatocytes cause cell toxicity (Kubitz et al., 2012). Many drugs which cause DILI inhibit BSEP

activity and it has been proposed that prospective screening for BSEP inhibition in drug discovery has the potential to aid early identification and deselection of drugs that have the propensity to cause human DILI, prior to clinical trials (Kenna et al., 2018).

Although various in vitro methods can be used to quantify inhibition of hepatocytes transporters (Shin et al., 2017; Du et al., 2017; Metry et al., 2022), currently it is unclear how the data provided by these assays can be used to assess the in vivo human DILI or DDI risk posed by investigational drugs. This is because the in vitro methods do not provide adequate insight into drug exposure or interplay of multiple processes as seen in vivo, while the biomarkers of liver toxicity evaluated in in vivo drug safety studies (plasma ALT and bilirubin in animals and humans, plus liver histopathology in animals) do not provide insight into the activity of hepatic transporters.

Currently, DDIs are assessed non-clinically via in vitro studies in a variety of cellular systems. Quantitative translation of in vitro data to in vivo is undertaken via physiologically-based pharmacokinetic (PBPK) modelling, which integrates in vitro transporter kinetic/inhibition data with relevant in vivo physiological parameters. PBPK models simulate changes in both systemic and tissue exposure of the victim drug, which arise as a result of changes in enzyme and/or transporter activity caused by the perpetrator drug [Guo et al., 2018; Galetin et al., 2017; FDA (A), 2018; FDA (B), 2020; FDA (C), 2020Guidance Document, 2020; PBPA Guidance for Industry).

In the development of an investigational drug (ID), PBPK analyses of such animal and cell studies often suggest that the ID may be a transporter inhibitor or inducer in man, and thus carry an enhanced DDI risk. In such cases, clinical studies of the impact of the perpetrator drug on plasma exposure of a victim drug are an early priority in drug development. Such a package of non-clinical and clinical studies is used routinely to support regulatory submissions and drug labelling and the value has been recognized in recently published DDI and PBPK regulatory guidance documents (FDA (A), 2018; FDA (B), 2020; FDA (C), 2020)[Guidance Document i, 2020; PBPA Guidance for Industry; Guidance Document ii, 2020].

However, verification of the accuracy of PBPK simulations is challenging, especially for transporter DDIs that arise due to changes in drug exposure within hepatocytes but not in plasma. Furthermore, clinical DDI studies are unable to detect DDIs which arise via inhibition of MRP2 and result in increased liver exposure to victim drugs, but not in detectable elevation of plasma drug concentration. Consequently, clinical trials have been shown to run the risk of either underestimating DDIs, and so potentially harming study subjects, or of failing to show sufficient drug efficacy because of overestimation of potential DDIs and poorly informed lowering of the drug dose ([FDA (A), 2018; FDA (C), 2020PBPA Guidance for Industry; Guidance Document ii, 2020; Rodrigues, 2019).

## 1.3 Hypothesis

To address this important drug development need, we are assessing the value of a widely available quantitative biomarker modality: dynamic gadoxetate enhanced magnetic resonance imaging (DGE-MRI). Currently the technology is used widely to characterise and diagnose human liver abnormalities.

Gadoxetate is excreted extensively by both liver and kidneys (Leyendecker et al., 2009). Hepatic excretion is known to occur via uptake into hepatocytes, which is mediated primarily via hepatocellular uptake by OATPs and to a lesser extent NTCP (Leonhardt, 2010), then MRP2-and MRP3-mediated excretion from hepatocytes into bile (Saito et al., 2013). With suitable dynamic MRI scan protocols, coupled to dedicated modelling of the data (see next section), gadoxetate liver uptake and excretion rates can be measured quantitatively. In principle, this novel liver imaging biomarker pair could be used in drug development to aid assessment of DDI risk arising from effects of test drugs on activities of transporters that mediate hepatocyte uptake and efflux of gadoxetate (i.e., OATP1B1, OATP1B3, NTCP, MRP2 and/or MRP3).

Previously, we and others have shown in rats that gadoxetate DGE-MRI can be used to assess and quantify kinetic rate constants for gadoxetate transport from the

extracellular space into hepatocytes (k(he)) and from hepatocytes into bile (k(bh)); and also to quantify the effects on the rate constants of 6 different test drugs which inhibit hepatobiliary transporters (Ulloa et al., 2013; Karageorgis et al., 2018; Georgiou et al., 2017; Scotcher et al., 2021; Melillo et al., 2023; Weinmann et al., 1996; Leonhardt et al., 2010; Jia et al., 2014).

In addition, we undertook a study in healthy volunteers given one of the test drugs (rifampicin). The study has shown a systematic 95% reduction in uptake and 40% reduction in excretion, which has provided proof-of-principle that the method can be used to quantify effects on human hepatocyte transporter function in vivo (Min et al., 2023). This work further ascertained gadoxetate, administered at 25% (¼) standard clinical dose, provides sufficient images on DGE-MRI to facilitate quantitative, kinetic assessment of DILI in those who had a single dose of rifampicin.

Since gadoxetate is not a substrate of BSEP, we do not consider that DGE-MRI will provide data that are likely to be informative of DILI risk, but instead is most suited to assessment of hepatocyte transporter mediated DDI risk.

## 1.4 The DGE-MRI assay

The specific structure of gadoxetate concentration-time profiles in the liver are reflective of the rate of liver uptake and excretion. There is typically a rapid initial uptake corresponding to the extracellular phase followed by a slower increase corresponding to intracellular uptake (Figure 2). With sufficiently long acquisition times, the curve shows a decrease due to biliary excretion. Biliary excretion typically occurs after approximately 20 minutes (commonly known as the hepatobiliary phase in diagnostic liver imaging). In diagnostic imaging this would produce the typical dynamic arterial, portal venous, transitional phase and hepatobiliary phase images as outlined in figure 3.

These diagnostic images are evaluated by visual inspection and do not produce quantitative measurements of gadoxetate uptake and excretion rates. In order to achieve quantification, rapid dynamic imaging is required to produce an accurate

assessment of the actual kinetic profiles, including rapid changes during the extracellular phase of the agent's distribution. For this reason, 3D images designed for quantitative analysis need to be acquired about every 3 seconds, which naturally comes with some penalty in terms of image quality. Moreover, since gadoxetate uptake and especially excretion are slow processes, this rapid imaging needs to be performed for at least 30 minutes, but significantly longer when liver function is impaired and gadoxetate elimination is correspondingly delayed. While 30 minutes may be adequate in a normal liver, our simulations have shown that, in order to detect a reduction of 90% in biliary excretion, images must be acquired over a time scale of at least 2 hours after administration of the inhibitor. Since this is not feasible in a single MRI scan, the protocol developed for this purpose involves two separate MRI scans, separated by approximately 60-90 minutes. This is not feasible for diagnostic purposes in clinical practice, but in early drug development where critical decisions are made, such studies are well justified.

The analysis of the images involves motion-correction of the MRI images to remove breathing motion, contouring of the liver and aorta to extract signal-time curves and MRi signal modelling to derive gadoxetate concentrations. These are then interpreted by fitting to a dedicated liver kinetic model (figure 4), producing the uptake rate khe and excretion rate kbh by fitting the model to the data. An example of the signal-time curves for aorta and liver, along with their model fits, is shown in figure 5. This example is taken from the proof-of-concept study with rifampicin in Leeds. The effect of rifampicin on the liver signal is clearly visible with the naked eye, showing a significant reduction in the signal after the initial extracellular phase. The quantitative analysis has revealed that this is explained by a 90% reduction in uptake rate, and 40% reduction in excretion.

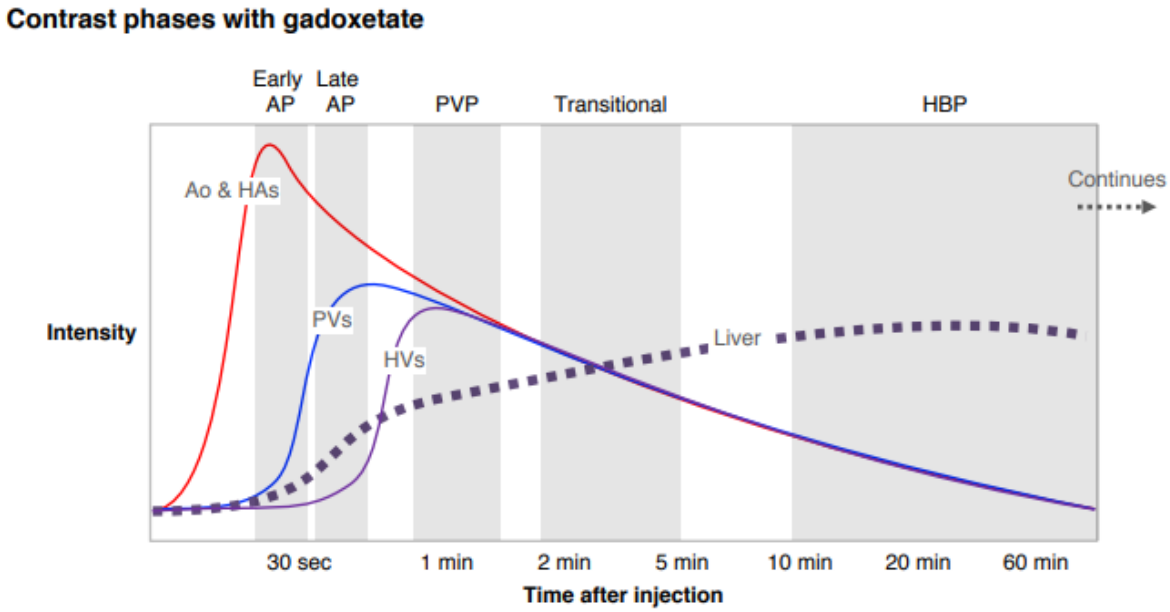


**Figure 2** - dynamic contrast phases with gadoxetate (Ao = aorta, HAs = hepatic artery, PVs = portal veins, HVs = hepatic veins, AP = arterial phase, PVP = portal venous phase, HBP = hepatobiliary phase)

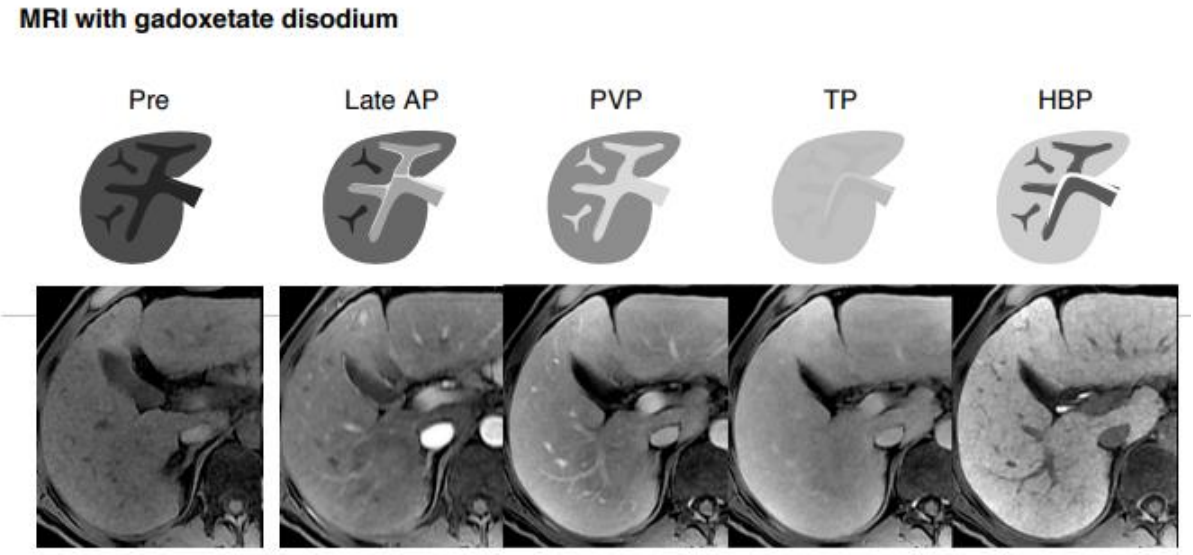


**Figure 3** - overview of MRI imaging phases pre and post gadoxetate

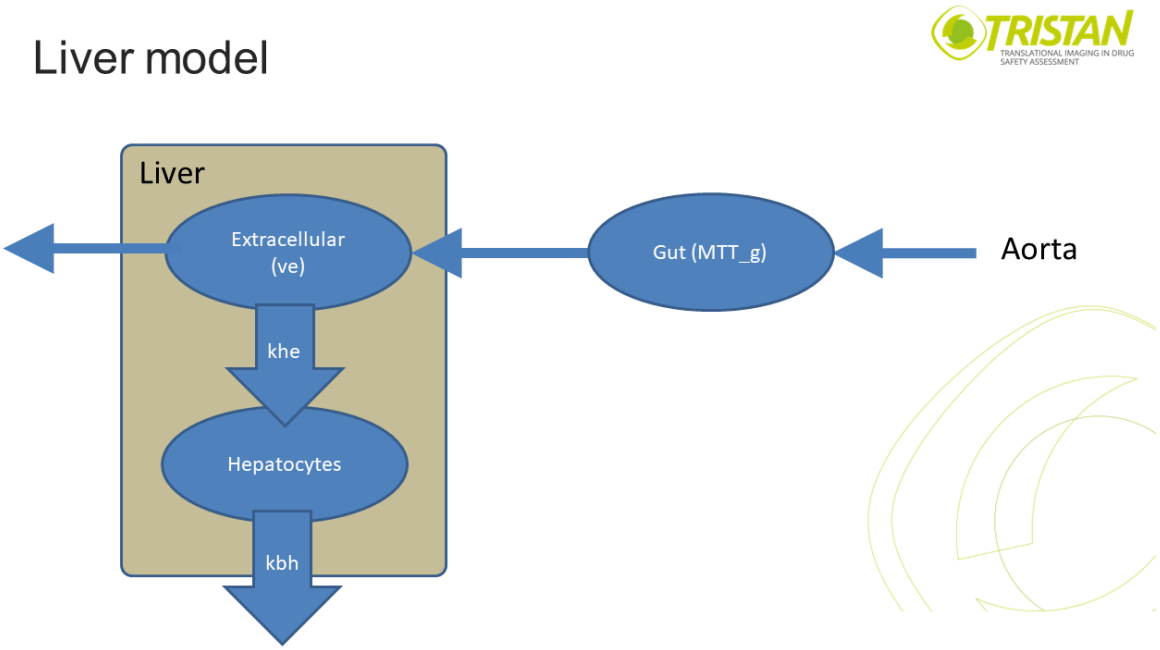


**Figure 4** – Diagram of the 6-parameter kinetic model used to derive gadoxetate uptake and excretion rates from rapidly sampled concentration-time curves. The model uses an aorta concentration as input, and further consists of one compartment for the gut, one compartment for the liver's extracellular space and one for the hepatocytes.

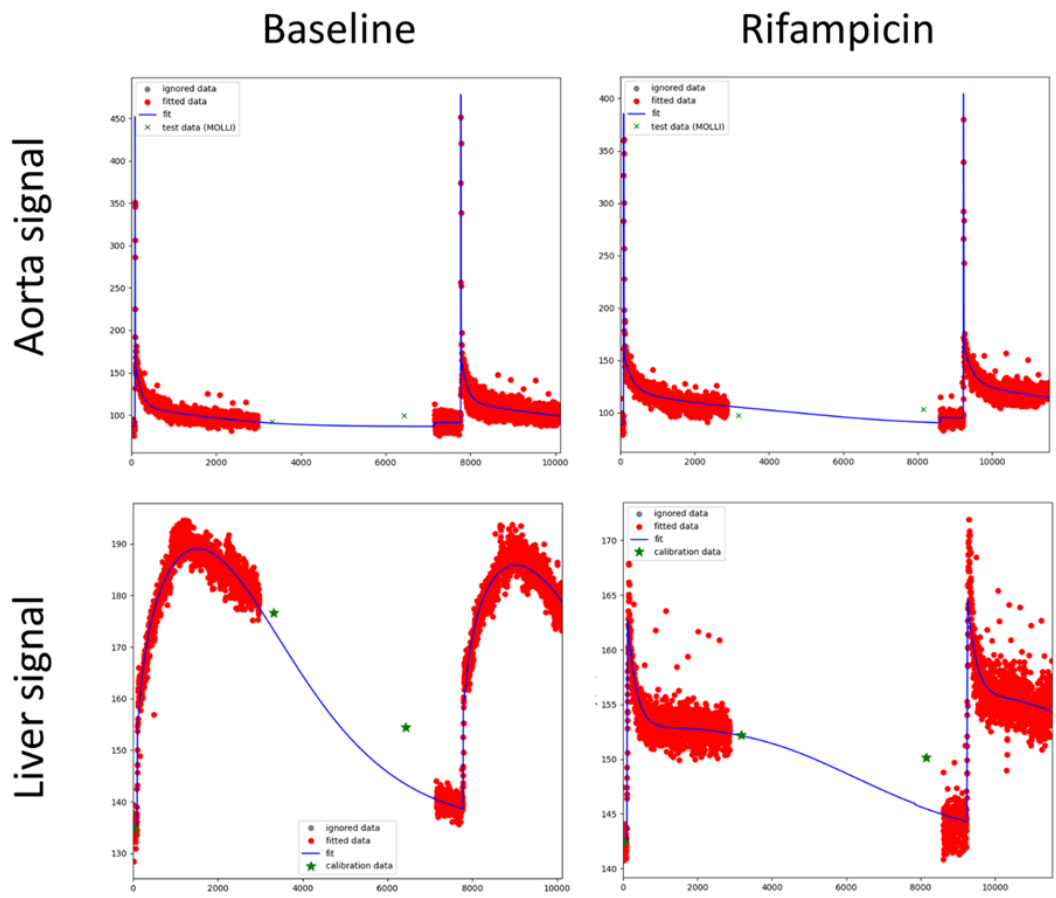


**Figure 5.** Signal-time curves along with model fits, for one volunteer of the proof-of-concept study at baseline (left column) and after rifampicin (right column) for the aorta (top row) and the liver (bottom row). Each plot shows the measured signal data for the two scans (red dots) and with the model fit (blue line). The X-axis shows the time since the start of the first scan in seconds.

# 2. Study Objectives

## 2.1. Study aims

The purpose of the current study is to expand on the proof-of-concept study with rifampicin and use DGE-MRI to measure the inhibition of gadoxetate uptake and excretion in volunteers with two other test drugs, metformin and ciclosporin (Neoral). These drugs are selected because they are commonly used in clinical practice, have a good safety profile and are known to inhibit OATP1B1 and MRP2 function (Ruan,

2023; Gertz, 2013). The results will add further evidence that these effects can be detected by DGE-MRI, improve our understanding of relevant effect size and limits of detection, and help us identify thresholds above which reduction in gadoxetate uptake or excretion would be of concern. In the longer term, establishing the sensitivity of gadoxetate rate constants would further strengthen our case that DGE-MRI can be used as a screening tool in drug development, to establish the impact of novel drugs on hepatic transporter function and the associated risk of DDI. Since DGE-MRI is a costly and time-consuming modality, a secondary objective of the study is to compare the effect size of the DGE-MRI biomarkers against that of standard liver function tests. If the results confirm that gadoxetate rate constants are more specific and more sensitive to drug effects than widely available LFT's, this would further strengthen the case for the use of DGE-MRI in this setting. The baseline values for the LFT's also act as an independent verification that the liver function of the study subjects is in the normal range.

Ciclosporin and metformin are chosen as test drugs because their kinetics is well characterised, they are commonly used in clinical routine and carry strong safety profiles.Ciclosporin is a potent inhibitor of hepatic uptake via interactions with OATP, in particular cyclosporin A is a time-dependent inhibitor of OATP1B1 with long-lastingness (Melillo et al., 2023; Izumi et al., 2015). In addition, ciclosporin is a potent inhibitor of MRP2 (Yang et al., 2020). Metformin does not interact with OATP, but instead with MRP2.; its Inhibition of MRP2 has been shown to impair biliary clearance of drugs such as atorvastatin, suggesting a possible mechanism for the unpredictable effects of statins on liver function. This impairs biliary excretion and increases liver concentration, without measurably affecting plasma concentration of the victim drug  (Shin et al., 2017).

Ciclosporin and metformin are commonly used in clinical practice and carry strong safety profiles. Neither metformin nor cyclosporin accounted for any adverse drug reactions as cause for hospital admission in a large, multicentre study, a finding which was reproduced in a more recent, smaller study (Pirmohamed et al. 2004; Osanlou et al. 2021). In a randomised double-blind clinical trial, no significant safety concerns were noted when using metformin in 18,000 patient-years of follow-up (The

Diabetes Prevention Programme Research Group, 2012). Similarly, low-dose cyclosporin has been shown to be safe, even when used long-term (Di Leo et al. 2011). When considering these studies were performed in patients on long term prescriptions with comorbidities, the safety data from these trials provides reassurance that they are safe drugs to use as a one-off dose in healthy subjects.

## 2.2. Endpoints

### Primary:

1. The effect of a clinical dose of metformin on hepatocellular gadoxetate uptake and excretion in healthy volunteers.
2. The effect of a clinical dose of ciclosporin (Neoral) on hepatocellular gadoxetate uptake and excretion in healthy volunteers.

### Secondary:

1. Compare effect size of gadoxetate uptake and clearance rates as measured by DGE-MRI with liver function test results in response to test drug administration.
2.The effect size of the drug on secondary imaging measures such as relative enhancement ratio or area under the curve in blood and liver concentrations.
3. Diurnal variations in uptake and excretion rates in the absence of an intervention (baseline data)

## 2.3 Long-term aim

On the longer term we expect these data to help build a case that DGE-MRI can be a useful tool to assess transported-mediated DDI risk in early drug development.

The TRISTAN consortium has been engaging with the FDAs biomarker qualification program for several years now, and in January 2021 reached a major milestone

when the FDA officially accepted the letter of intent (LoI) outlining the definition, assay and intended use of gadoxetate uptake and excretion rates as a biomarker for drug safety studies (https://www.fda.gov/media/149415/download).

In December 2023 the TRISTAN consortium submitted a biomarker qualification plan (BQP), describing a collection of experiments that have been and will be performed to support a full qualification application. The BQP included the proof-of-concept study in Leeds, a currently ongoing patient study, and the 2-compound study described in this protocol. In august 2024 another major milestone was reached when the FDA formally classified the BQP as reviewable, meaning the agency will invest time and resources for a detailed review and feedback within 6 months.

After revision and final acceptance of the BQP, the final step in the process will be a full qualification submission. The results derived in this study will be included in that submission as a critical step towards formal approval of these novel biomarkers for use as a drug safety marker in early stage drug development.

# 3. Design summary

This study is an observational study of gadoxetate-enhanced MRI as a putative imaging biomarker of drug transporter function in the liver. The overall design is summarised in Figure 1.

All study procedures will be done at Sheffield Teaching Hospitals NHS Foundation Trust at the Royal Hallamshire Hospital (RHH) site. This is the site for University of Sheffield MRI related research. All scanning will be performed on the 3T University MRI scanner and images will be anonymised and transferred securely to the University of Sheffield network for analysis. Participant understanding and consent will be checked at each visit.

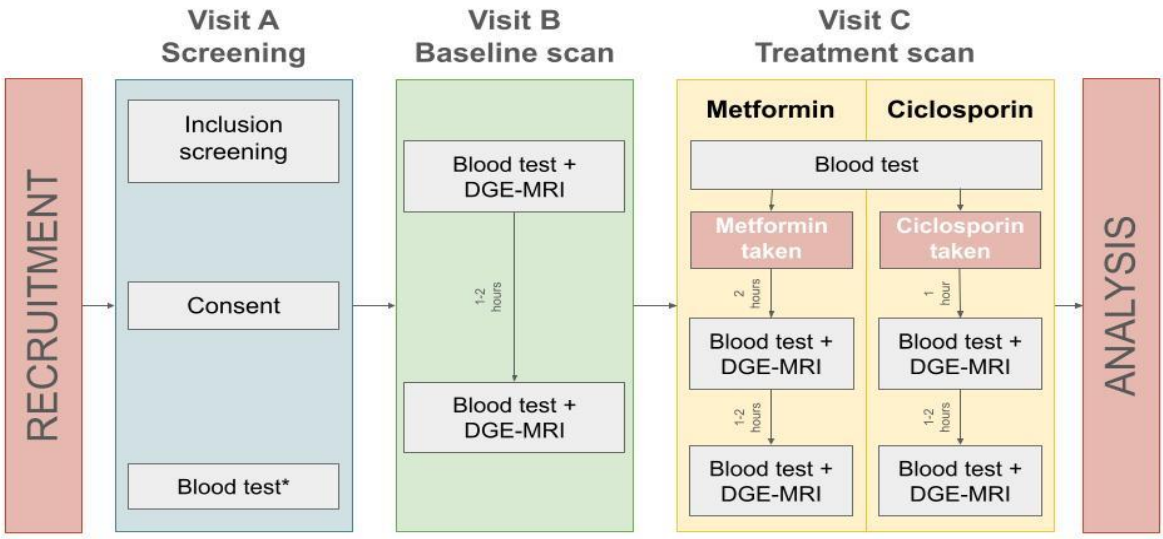


**Figure 1.** *Study flow diagram.*

## 3.1 Recruitment

Six healthy volunteer participants will be recruited to each drug arm (3 men and 3 women for each drug). Participants will be recruited via an advert on the internal University of Sheffield Medical School platform 'Minerva' and on callforparticipants.com. Participants will be recruited on a first come first serve basis with only a contact email collated at this point. Following email confirmation of their interest by the research fellow, the participant information sheet (PIS) will be provided for them to read, informing them of the risks and benefits of the study, and the requirements of them throughout. A unique Study ID will be generated for the participant. A further screening form will be sent to the participant which will ascertain their eligibility to participate and collate any pertinent information. The form will be associated with their unique Study ID and no personal nor identifiable information will be collected.

## 3.2 Visit A - Screening/consent visit

Eligibility screening will be performed through the verification of their responses. The PIS will be reviewed with the participant in detail to ensure they understand the risks and benefits of the study along with the requirements of them. A consent form will be

completed. Participants will undergo clinical examination with observations (respiratory rate, oxygen saturations, blood pressure, heart rate, temperature). A blood test to assess renal and liver function will be taken. If the renal function blood test shows eGFR<30mL/min/1.73, markers of liver function exceed laboratory normal values or if the clinical examination does not meet inclusion criteria, the participant will be removed from the study and will not be counted towards the study target. Blood markers of liver function for this study are aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT) and bilirubin. Any abnormal results will be communicated to the participant and with their permission, the participant's general practitioner.

## 3.3 Visit B - Baseline visit

Two DGE-MRI scans will be performed with gadoxetate given at 25% (¼) standard clinical dose. The duration of each scan will be approximately 60-75 minutes. There will be a between-scan interval break for the participants of between 60 and 120 minutes.

The research fellow, prior to the scan, will insert an intravenous cannula to the participant's arm to draw blood samples (5mL each) for liver function before each scan, and to administer a contrast agent during the scan.

After the baseline visit and before the next visit, the scan data will be analysed, specifically assessing image quality and implementation of the PBPK model of gadoxetate kinetics. Two outcomes are possible:

1. The image quality and PBPK modelling is satisfactory. The participant will proceed to Visit C within 56 days and have DGE-MRI scanning repeated following metformin or ciclosporin (Neoral) administration.
2. The image quality or PBPK modelling is insufficient. In this case, the cause of this will be reviewed by the senior research team and the participant will be removed from the study.

## 3.4 Visit C - Treatment visit

Participants will now enter the arm of the study which they were recruited for, to receive either metformin or ciclosporin (Neoral). They will undergo two DGE-MRI scans using the same dosage of gadoxetate and imaging protocol used in Visit B. Participants will have a set of observations and a clinical examination performed before administering the medication and be excluded if found to have uncontrolled hypertension or an active infection.

Participants will receive either a single oral dose of 1000mg immediate-release metformin or 100mg ciclosporin (Neoral). Ciclosporin (Neoral) will be given 1 hour before gadoxetate administration. Metformin will be given 2 hours before gadoxetate administration. Following this initial scan, the participant will be given a 60-120 minute break after which a second DGE-MRI scan will be performed. Each scan will take 60-75 minutes with a maximum total scan time of 150 minutes at this visit.

The research fellow will perform venepuncture on arrival, before metformin or ciclosporin (Neoral) administration, for a liver function blood test. Prior to each scan the research fellow will insert an intravenous cannula to the participant's arm for the administration of contrast agent. At each insertion, a blood sample for liver function will be taken (totalling 3 blood tests at this visit, or 5mL each).

Total dosage of gadoxetate administered across all visits is 100% standard clinical dose and will not be exceeded.

## 3.5 Monitoring procedures

There will be no participant monitoring procedures undertaken following completion of the Visit C but the research fellow will remain contactable for further queries.

# 4. Sample size estimation

The proof of concept study at the University of Leeds performed previously in this research stream used a minimum sample size of 6 participants. This matched the sample size of previous animal studies in which the reduction of hepatic gadoxetate uptake following rifampicin administration was approximately 50% compared to the control.

Using data from the human proof of concept study, a new analysis showed that the minimal number of subjects needed to demonstrate an inhibition in uptake and excretion of 20% or more (a typical cutoff for DDI risk in drug development) with 95% confidence was 4 subjects per drug. The number was increased to 6 per drug to allow for drop-outs after the first visit or scan failures (which happened in 3/20 scans in the pilot study) and allow for an equal number of men and women per drug.

# 5. Participant Selection Criteria

## 5.1 Inclusion criteria

- Able to provide informed consent
- Age above 18 years
- Able to attend for all scheduled visits
- Able to adhere to instructions on pre-scan food and drinks intake
- Able to lie flat for the duration of the scan

## 5.2 Exclusion criteria

Standard MRI exclusions, including:

- No history of liver or kidney disease
- No history of heart failure
- No history of uncontrolled hypertension
- No history of malignancy or premalignant conditions, past or present
- No current uncontrolled infections

- Not currently pregnant
- Not currently breastfeeding
- No regular prescribed medication, except the oral contraceptive pill
- Cochlear implant
- Aneurysm clips
- Neurological stimulator
- Implanted cardiac devices (ICD, PPM, loop recorders, or any others)
- Metal heart valve
- History of retained metal foreign bodies
- Other implanted metal device which prevents MR imaging
- Claustrophobia
- Weight exceeding 140 kg
- History or allergic reaction to MRI contrast agent

# 6. Participant Procedures

## 6.1 Overview of study visits

Participants will undergo study visits as outlined below and in Figure 1:

- Recruitment
- Visit A - Pre-Screening/Consent visit with pre-scan renal function blood test
- Visit B - Baseline scan visit
- Visit C - Treatment scan visit

All study visits will be face-to-face at Sheffield Teaching Hospitals, RHH site and DGE-MRI scans will be performed on the University of Sheffield MRI scanner at the same site. Understanding of the research procedures will be checked at each visit and consent taken again.

## 6.2 Recruitment

Participants will be recruited via two methods. An advert will be placed on the University of Sheffield Medical School internal platform 'Minerva' which medical students and staff use. There will be a link to an online form to complete which will confirm they have read the entry requirements and will collect only their email. A further advert will be placed on www.callforparticipants.com with the same information and will again confirm that the participant meets the entry requirements and collect their email.

The email of the research fellow will be on the advertisement for any participants wishing to contact the team directly. After expressing interest, the participant will receive an email from a research fellow inviting them to a screening visit and will be asked to respond with a suitable time. If a participant does not respond initially, a further email will be sent. If there is no response following this email, the participant will be removed from further consideration and their email will be deleted.

On agreeing an appointment for the screening visit, a unique Study ID will be generated for the participant. The research fellow will provide the participant with the PIS and will ask the participant to study this prior to the agreed meeting. Participants will be given at least 72 hours to consider the information in the PIS before the meeting. A further screening online form will be sent to the participant which will ascertain their eligibility to participate and collate any pertinent information. The form will be associated with their unique Study ID and no personal nor identifiable information will be collected.

Participants will be remunerated by a £150 flat-rate given to participants after completion of all scans and £75 after completion of one scan visit. A standardised, free lunch will be given to participants, with flexibility to adapt to any dietary requirements. In addition, travel expenses will also be reimbursed up to the amount of £50. All expenses will be paid in cash. This will be on the advertisement and in the PIS.

## 6.3 Visit A - Screening/Consent visit

A research fellow will verify the responses of the screening online form with the participant face-to-face to ensure the participant meets eligibility criteria and is safe to proceed. Female participants of child-bearing potential will have a urine pregnancy test performed to detect human chorionic gonadotropin (HCG) in their urine. A negative HCG result will confirm a non-pregnancy status and will be used to exclude unsuspecting pregnant participants from enrolling onto the study.

The research fellow will then explain the study in detail and review the PIS with the participant, outlining that progression through the study is not guaranteed dependent on biochemical and imaging results. This will be done in accordance with the requirements of the ethics review and human subject research regulations. The written comprehensive consent documents will be reviewed. Participants will be asked to provide consent for the blood tests and MRI scans as well as to store and share their anonymised MRI images for future research. Written consent will be obtained.

The research fellow will take a set of observations (respiratory rate, oxygen saturation, blood pressure, heart rate and temperature) and perform a clinical examination. Participants will be excluded if found to have uncontrolled hypertension or active infection. The research fellow will also perform venepuncture to assess the participant's renal and liver function. If the renal function blood test shows eGFR<30mL/min/1.73, markers of liver function (as outlined in 3.2) exceed laboratory normal values or if the clinical examination does not meet inclusion criteria, the participant will be removed from the study and will not be counted towards the study target. The abnormal blood test or clinical examination will be communicated to the participant and with their permission, the participant's general practitioner.

The screening/consent visit will last no more than 30 minutes with an addition of 10 minutes if blood sampling is done in the same setting.

On completion of the above, dates for Visit B and Visit C will be agreed.

## 6.4 Visit B - Baseline Scan

Participants will undergo two DGE-MRI scans and two blood tests during this visit. Participants will be asked to attend having fasted for at least 4 hours before the time of the scan. Participants will be counselled to only drink water prior to the scan. An MRI screening form will be completed in accordance with local practice. Participant's height and weight will also be recorded. Female participants of child-bearing potential will have a urine pregnancy test performed to detect human chorionic gonadotropin (HCG) in their urine. A negative HCG result will confirm a non-pregnancy status and will be used to exclude unsuspecting pregnant participants from the risk of the dye. Pregnancy tests will be provided .

A research fellow or experienced radiographer will insert an intravenous cannula into the participant's arm for the administration of contrast agent during the examination. At this time, a blood sample will be taken (5mL) and sent for assessment of liver function.

There will be two DGE-MRI scans with a gadoxetate injection at 25% (¼) standard clinical dose at each. Gadoxetate will be injected at 60 seconds into the first scan acquisition and at 10 minutes into the second. Each scan will take approximately 60 to 75 minutes with a maximum total scan time of 150 minutes at this visit. There will be an interval break for the participants of between 60 and 120 minutes in which a standardised lunch will be provided.

The participant will be asked to remain still on his/her back throughout the duration of the scan. The staff will maintain constant communication with the participants in between image acquisitions. Participants will be able to decide to stop and withdraw from the scan at any time. If this is the case, scanning will be stopped and the participant will be removed from the study.

## 6.5 Visit C - Treatment scan

Participants will be asked to attend having fasted for at least 4 hours before the time of the appointment. An MRI screening form will again be completed in accordance with local practice. Participant information including height and weight will also be recorded. Participants will have a repeat set of observations (respiratory rate, oxygen saturation, blood pressure, heart rate and temperature) and a clinical examination. Participants will be excluded if found to have uncontrolled hypertension or an active infection. Female participants of child-bearing potential will have a urine pregnancy test performed to detect human chorionic gonadotropin (HCG) in their urine. A negative HCG result will confirm a non-pregnancy status and will be used to exclude unsuspecting pregnant participants from the risk of the dye and drug. Pregnancy tests will be provided.

A blood test for liver function will be taken via venepuncture by the research fellow before administering the drug. Before each scan, the research fellow or experienced radiographer will insert an intravenous cannula to the participant's arm for the administration of contrast agent during the examination. At this time a blood sample will be taken (5mL) for liver function tests using the same cannula (totalling 3 samples, 15ml).

The participants will take either 1000mg immediate-release metformin or 100mg ciclosporin (Neoral) orally with water and remain fasted until the MRI scan. For ciclosporin, gadoxetate will be administered 1 hour after ingestion. For metformin, gadoxetate will be administered 2 hours after ingestion. Two DGE-MRI scans will be performed with a gadoxetate injection at 25% (¼) standard clinical dose at each. Gadoxetate will be injected at 60 seconds into the first scan acquisition and at 10 minutes into the second. Each scan will take approximately 60 to 75 minutes with a maximum total scan time of 150 minutes at this visit. There will be an interval break for the participants of between 60 and 120 minutes.

The participant will be asked to remain still on his/her back throughout the duration of the scan. The staff will maintain constant communication with the participants in

between image acquisitions. Participants will be able to decide to stop and withdraw from the scan at any time.

Participants will not receive greater than a standard clinical dose of gadoxetate in total across both Visit B and C of this study.

# 7. Data and Sample Collection

## 7.1 Preparation

Volunteers will be required to prepare for each scan as follows:

- 72 hours prior to the day of the scan
    - Abstain from drinks containing alcohol
- On the day of the scan
    - Fast for 4 hours before Visit B and C

## 7.2 Clinical information

The following information will be acquired prior to each scan:

- Documentation of:
    - Height
    - Weight
- Standard MR screening form

## 7.3 Blood Samples Collection and storage

Blood samples will be taken for screening renal function and liver function taken during screening (Visit A), baseline (Visit B) and treatment (Visit C) visits as outlined in 6.4 and 6.5 respectively. All of the plasma samples will be destroyed when the study ends. Blood samples for liver and renal function will be processed routinely as per Sheffield Teaching Hospitals laboratory policy.

## 7.4 MRI scanning

The lead investigator will coordinate with the radiography team who operate the University of Sheffield MRI scanner to book slots for participants and inform them of the protocol. The research fellow will liaise with participants and the radiography team to organise scanning as per the protocol. Up to 30 breath-holds of up to 12 to 17 seconds each may be required. There will be periods of free breathing scans between different breath-hold scans, giving the participants several rest periods. The protocol is specified in detail in SOPs provided and maintained by TRISTAN partner Bioxydyn, including quality assurance measures and radiographer training.

# 8. Data analysis

Analysis of MRI data will be done according to the study design. The imaging data will be quality reviewed scan by scan after Visit B to ensure that adequate scans are being obtained for quantitative analysis.

Images will be processed by the research team on encrypted University of Sheffield computers using custom-written software to perform breathing motion correction, and segment the liver and aorta. Signal-time curves will be extracted as csv files and processed using custom-made python scripts to extract measurements of gadoxetate uptake rate (khe, mL/min/100mL) and gadoxetate excretion rate (kbh, mL/min/100ml) for each subject and for each visit. An effect size will be determined for each subject and for each rate constant. This will serve as main outcome measures:

Effect size (%) = 100* (rate constant with drug - rate constant without drug)/(rate constant without drug)

Statistical analysis of gadoxetate kinetics will only commence after all the scans in Visit C have been completed.

## 8.1 Outcome measures

### 8.1.1 Primary outcome measures

The primary outcomes of interest are to:

1. 95% CI on the mean effect size of uptake rate khe
2. 95% CI on the mean effect size of excretion rate kbh

Gadoxetate kinetics will be described under a bespoke and previously validated physiologically-based pharmacokinetic (PBPK) model where both uptake and efflux rates can be calculated. Additional imaging parameters will be described to mirror the previous animal and human studies, including the relative enhancement of the liver following gadoxetate administration and T1 values pre and post gadoxetate administration.

### 8.1.2 Secondary outcome measures

The secondary outcomes of interest are:

1. Compare effect size of gadoxetate uptake and clearance rates as measured by DGE-MRI with liver function test results in response to test drug administration.
2. The effect size of the drug on secondary imaging measures such as relative enhancement ratio or area under the curve in blood and liver concentrations.
3. Diurnal variations in uptake and excretion rates in the absence of an intervention (baseline data)

## 8.2 Statistical analysis

The average effect size of all 6 subjects will be determined for each drug, as well as the 95% confidence interval on the mean (mean +/- 1.96*sdev/sqrt(6)). The drug will be considered to pose a DDI risk if the 20% threshold falls outside of the 95% CI for mean khe or kbh effect size.

An independent analysis of the findings will be conducted by Bioxydyn, the study collaborator.

# 9. Risks

There are a number of risks of differing likelihoods associated with undertaking this observational study which are outlined below. All this information will be made available to participants prior to enrolling in the study.

## 9.1 MRI scanning

MRI scans do not involve any radiation and are safe and painless. There are no known risks to this technique as long as standard MR exclusion criteria are satisfied. Some people may experience claustrophobia in an MRI scanner. The scan can however be stopped at any time, at the participant's request. The MRI staff will monitor the participant via a video camera and an audio link. The participant can also pull out of the study or scan at any time. Participants will be required to do breath holds as standard MRI scanning protocol to improve quality of images.

## 9.2 Contrast agent and medications

Allergic reactions are rare but possible in gadoxetate, metformin and ciclosporin. Equipment and treatments are immediately available at RHH should a reaction occur with an on-call medical team on site. Specific risks associated with each are outlined below.

### 9.2.1 Gadoxetate (Primovist®)

Gadoxetate is an intravenous contrast agent used extensively in clinical imaging studies of the liver. Gadoxetate is safe but there are a number of known adverse effects. Headache and nausea are commonly reported but are transient. A feeling of breathlessness is also reported at a rate of approximately 1 in 100 persons administered this contrast agent. It should be used with caution in those with renal impairment, due to the risk of nephrogenic systemic fibrosis. Therefore, a pre-scan renal function blood test will be performed during the study. If the renal function

blood test shows eGFR<30mL/min/1.73, the participant will not be eligible for the study. The incidental abnormal results will be communicated to the participant and with their permission, the participant's general practitioner.

Gadoxetate is a gadolinium salt and there are reports of accumulation of gadolinium in the brain of persons administered this agent (Murata, 2016(A); Murata, 2016(B)) There are no reports of any clinical sequelae as a consequence of this accumulation and gadoxetate is widely used in clinical practice for the assessment of focal liver lesions.

Gadoxetate will be used at 25% (¼) standard clinical dose per scan, meaning that with a maximum of four MRI examinations, each person will receive 100% of a standard clinical dose overall. This will not be exceeded and if imaging is not sufficient for kinetic analysis, the participant will not undergo any repeat imaging and be excluded from the study.

The administration of gadoxetate will be authorised by the clinical lead, Dr Benjamin Rea.

### 9.2.2 Metformin

Metformin is an antihyperglycaemic medication authorised for use in the treatment of type 2 diabetes mellitus. It is given orally and comes in immediate-release and modified-release preparations. The immediate-release preparation starts at a dose of 500mg and can be uptitrated to 2g daily in three divided doses, taken at meal times. The modified-release preparation starts again at a dose of 500mg and can be uptitrated to 2g in two divided doses, at morning and evening meals. This study will use 1000mg immediate-release metformin to match previous literature on metformin DDIs (Hibma, 2016).

Metformin is a well established diabetic medication that has been used clinically for over 50 years. Adverse effects of treatment are known and are more commonly experienced with immediate-release preparations and if not taken with food. Common adverse effects include abdominal pain, decreased appetite, diarrhoea,

nausea, altered taste, vitamin B12 deficiency and vomiting. These side effects are most common on initiating the medication and resolve spontaneously on cessation. More rare and severe adverse reactions including skin reactions, hepatitis and lactic acidosis. The manufacturer advises caution in those at risk of lactic acidosis including those with chronic stable heart failure and other medications that are known to impair renal function. Therefore, inclusion criteria requires participants to have no history of heart failure and no regular prescribed medications.

Metformin has been reported to inhibit MRP2 activity in vitro and in rats in vivo (Shin et al., 2016). In a rat DGE-MRI study, metformin administration reduced the rate of gadoxetate efflux from the liver but did not alter the rate of gadoxetate uptake, indicating functional inhibition of MRP2 but not OATP transport activity in vivo (Melillo et al., 2023).

However, overall metformin has an excellent safety profile. In two large studies looking at the medications responsible for patients being admitted with adverse drug reactions (ADR), metformin was not responsible for any of the 1443 ADR related admissions (Pirmohamed et al., 2004; Osanlou et al., 2021). The first study (Pirmohamed et al., 2004) in particular looked at 19920 patient admissions, and the two major risk factors for ADR identified from these studies were polypharmacy and multimorbidity. Given our proposed study would by using healthy volunteers who other than the OCP would not be on any regular medication, any risk to the participants would be mitigated. Additionally, given Metformin was not responsible for any ADR related admission despite being a commonly prescribed medication in patients that often have established risk profiles (cardiovascular disease, obesity, metabolic syndrome) the risk to a healthy volunteer participant from a one of dose is negligible.

### 9.2.3 Ciclosporin

Ciclosporin is an immunosuppressant medication authorised for use in the treatment of many immune-mediated diseases such as rheumatoid arthritis, psoriasis, inflammatory bowel disease and to prevent rejection in organ transplantation. It can

be administered orally or intravenously. The dosage is calculated based on the body weight ranging from 1.25mg/kg to 15mg/kg as single or divided doses depending on the indication. This observational study will use a standardised dosage of 100mg. This dose was determined to match previous literature on ciclosporin DDIs (Kajosaari, 2005). This will be in the Neoral formulation as others have different bioavailability and different DDIs.

Contraindications include malignancy, uncontrolled hypertension and uncontrolled infections. These will be screened for before enrollment and participants will have a set of observations (respiratory rate, oxygen saturation, blood pressure, heart rate and temperature) and a clinical examination performed before administering the medication in Visit C. Those with uncontrolled hypertension and active infection will be excluded at this point.

Common adverse effects include eye inflammation, decreased appetite, diarrhoea, electrolyte imbalance, fatigue, fever, flushing, gastrointestinal discomfort, gingival hyperplasia, hair changes, headaches, hyperglycaemia, hyperlipidaemia, hypertension, hyperuricaemia, leucopenia, muscle complaints, nausea, paraesthesia, peptic ulcer, renal impairment (renal structural changes on long-term administration), seizure, skin reactions, tremor, vomiting. Uncommon and rare adverse effects include anaemia, encephalopathy, oedema, thrombocytopenia, increased weight, gynaecomastia,haemolytic anaemia, idiopathic intracranial hypertension, menstrual disorder, multifocal motor neuropathy, muscle weakness, myopathy and pancreatitis.

Ciclosporin is reported to be a potent in vitro inhibitor of multiple hepatocyte uptake and efflux transporters, which include both OATP1B and MRP2 (Izumi et al., 2015; Yang et al., 2020). In a rat DGE-MRI study, ciclosporin administration markedly reduced the rate of gadoxetate uptake into the liver and also biliary excretion from the liver, demonstrating functional inhibition of both OATP and MRP2 transport activity in vivo (Melillo et al., 2023).

Ciclosporin is a long established immunosuppressant medication. In the aforementioned large UK based studies analysing admissions due to ADR, as with

Metformin, Ciclosporin was not attributed to be the cause of any ADR admission. (Pirmohamed et al., 2004; Osanlou et al., 2021). As an immunosuppressant Ciclosporin necessitates an excellent safety profile, and therefore the risk posed to a healthy volunteer with no history of polypharmacy or comorbidities from a one off dose, is once again negligible.

### 9.2.4 Cannulation and Venepuncture

Intravenous cannulation would be required for administration of gadoxetate prior to MRI scan. This will be done by an experienced radiographer or research fellow. Where possible, a blood sample will be taken off a cannula on initial insertion. For subsequent blood sampling, venepuncture will be required as blood sampling from a used cannula will cause erroneous results and increase risk of cannula-related infection. This will also be done by an experienced radiographer or research fellow. A blood test will check for pre-scan renal function blood test to ensure that this is normal due to the risk of nephrogenic systemic fibrosis on administration of gadoxetate in those with renal impairment. If the renal function blood test shows eGFR<30mL/min/1.73, the participant will be excluded from the study. Additional blood tests will check for liver function. The cannula will only remain in situ for the MRI scan. After the cannula removal or following venepuncture, there may be bruising and discomfort that may persist for a few days which should settle spontaneously.

There is a small risk of needlestick injury which will be minimised by the experienced radiographer or research fellow following local sharp safety measures. If a needlestick injury were to occur, local policy on initial actions will be followed and the occupational health department will be contacted to advise on further measures required.

## 9.3 Incidental findings

To look for incidental findings, images will be reviewed by Dr Rea, a consultant in gastrointestinal and abdominal radiology. Any abnormal blood results will further be reviewed by a research fellow using local lab parameters. For any unexpected abnormal finding related to the MRI scan, this will be reviewed by an expert liver

radiologist (Dr B Rea). If necessary, a clinical report will be issued. All clinically relevant incidental findings will be communicated back to the participants' General Practitioner with the participants' permission . In rare cases, the results will also be communicated to a clinical hepatologist. This process will be discussed and explained to the participant during the consent visit.

## 9.4 Nosocomial infections

There is a risk of hospital-acquired (nosocomial) infections upon attending RHH. These infections are commonly viral in nature, most likely a seasonal virus (causing the common cold) or SARS-CoV-2 (COVID-19). The morbidity and mortality associated with COVID-19 has significantly decreased from its peak in early 2021. Both public health and local hospital restrictions have eased accordingly. Other viral infections such as influenza show yearly seasonal peaks in Autumn and Winter (UKHSA, 2024). We will closely adhere to national Public Health England and local Sheffield Teaching Hospitals infection control policies to minimise the risk of hospital-acquired infection for the participants. Precautions include but are not limited to: regular hand washing, frequent surface decontamination and ventilation.

Visits will be limited to one participant per session and participants will be allocated a pre-secured MRI time slot to avoid unnecessary waiting or infective contacts. The clinical areas used at RHH do provide acute inpatient services but for the purposes of this study, participants will be confined to the radiology department, reducing potential exposures.

The cohort of participants are healthy volunteers, aged 18 years or over without other health concerns. This may reduce the additional risk of contracting or developing a severe infection.

## 9.5 Urgent Safety Measures

The Sponsor or Chief Investigator may take appropriate urgent safety measures with the intention of protecting research participants against any immediate harm to their health or safety.

The event will be reported within three days to the Research Ethics Committee (REC), that such measures have been taken, the indication for such measures and plan for further actions.

## 9.6 Safety Reporting of Serious Adverse Events (SAEs)

A serious adverse event (SAE) is defined as an untoward occurrence that:

- results in death;
- is life-threatening;
- requires hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity; or
- consists of a congenital abnormality or birth defect; or
- is otherwise considered medically significant by the investigator.

Any adverse events relating to this study will be reported and managed internally at Sheffield Teaching Hospitals. In the unlikely scenario that a SAE is unexpected and is felt to be related to a study procedure, the event will be reported to the Research Ethics Committee. The SAE will be reported to the sponsor within 1 working day of the research team's awareness of the event. To alert the sponsor, a serious adverse event reporting form (see appendix 13.1) will be completed and reported to the Director of Research, Partnerships & Innovation and Research Ethics & Integrity Manager whose contact details are available here.

Furthermore, REC will be made aware within 15 days of the Principal Investigator becoming aware of the event and will be reported through the non-CTIMP safety report to REC form available here (see appendix 13.2).

# 10. Study documentation

## 10.1 Study protocol

The protocol describes the background and purpose of the study, explains which procedures will be done and why, and details how the results will be utilised and interpreted.

## 10.2 Recruitment advertisement

The same advert will be posted on Minerva and callforparticipants.com as outlined in 6.2. The advert will outline the study, the participants role and the eligibility criteria. Potential participants will be asked to confirm they have read the eligibility criteria and enter their email address to allow the research fellow to contact them. No other information will be collated at this point.

## 10.3 Screening questionnaire

The screening questionnaire is filled in by a research fellow during Visit A (screening/consent visit) to confirm eligibility of the participant for the study. Questions relate to MRI safety, general health and past medical history. The questionnaire *does not include any identifiable information* about the potential participant. If, at the end of the screening visit, the participant declines to volunteer for the study, the questionnaire will be destroyed. On giving consent for inclusion in the study, the participant's questionnaire will be marked with their unique Study ID for future reference.

## 10.4 Participant Information Sheet (PIS)

The Participant Information Sheet contains information on the study relevant to an interested participant. It explains the purpose of the study, what is expected of the participant and the associated risks and benefits. It is given to the participant on agreeing a date to complete Visit A (screening visit), at least 72 hours beforehand. During the screening visit, the participant may ask any questions about the study and address any concerns, allowing them to make an informed choice on volunteering for the study. During the course of the study, the PIS will also serve as a personal record for each volunteer, showing their Study ID and details of their completed and upcoming scans.

## 10.5 Consent form

At the time of the screening visit, once all study details have been explained and understood, the participant will be asked to sign a consent form. This will be written (not electronic) consent. Through the above measures and the PIS, the participant

will make an informed decision on their inclusion in the study. Written consent will be obtained at each visit to ensure understanding is maintained.

## 10.6 MR screening form

These questionnaires review standard MR exclusion criteria as outlined in 5.2. They will be given to the participant by the site radiographer and will be stored according to local procedures using anonymised study IDs.

# 11. Data management

All data collated or generated during this research study will be stored short term on an encrypted shared Drive (administered by university IT) with only the investigators and research fellows having access. This data may need to be analysed on systems such as PACS or proprietary software for incidental findings assessment, liver segmentation and gadoxetate kinetic assessment but data will be removed from these systems on completion of the relevant analysis.

Upon completion of the research activities all data will be deleted from this short term storage and will be stored, fully anonymised, in a long-term repository as outlined in 11.5.

## 11.1 Confidentiality and personal data

Volunteers will be assigned an anonymous, unique Study ID after consenting to be included in the study. All data collected for research purposes will only be linked to their Study ID. Other data captured on the scanner includes date of birth, height and weight. This data will be handled securely in line with local practice. The link between anonymous study ID and the participants real identity will be stored exclusively in a single key file that will be stored securely on STH premises.

### 11.1.1 Recruitment forms

The online form used to collate participants via Minerva will be created by a research fellow stored on the shared encrypted University of Sheffield Google account and will

only be accessible by those in the research group.  No personal information and only an email will be collected to allow the research fellow to contact those interested.

A callforparticipants.com advert will be used which does not have access to participant responses and no data will be sent to third parties.  No personal information and only an email will be collected to allow the research fellow to contact those interested.

### 11.1.2 Screening questionnaire

Following email confirmation of a participant's interest to be included, a Study ID will be generated for them.  A further online form will be provided to participants to screen for their eligibility prior to Visit A.  No personal identifying information will be collected and all responses will be associated with the participants Study ID.  This document will be digital only and stored on the shared encrypted University of Sheffield Google Drive account.

### 11.1.3 Consent form

Participant written consent forms will contain their names and signatures. On the day of the study, these forms will be securely stored in the University MRI scanner room (with restricted entry) at the RHH site. The Sheffield Teaching Hospitals radiology department and University MRI suite is of restricted entry with swipe card access requiring appropriate security clearance. At the end of each visit, the forms will be scanned and uploaded to the encrypted, shared research group Sheffield Teaching Hospitals  Drive.  All paper copies will be archived for 5 years within STH premises.

### 11.1.4 MR screening forms

MR screening forms will be stored locally in accordance with local clinical practice. All paper copies will be archived for 5 years within STH premises.

### 11.1.5 Key file

A digitally encrypted key file, linking Study ID to participant names, date of birth and contact details, will be stored on the local STH drives, separate from all other study data. The key file will be securely stored for 5 years at the end of the study, after all

MRI scans on all participants have been completed. In the event that a participant withdraws from the study and requests their data and information to be removed, their entry in the key file will be used to identify and remove their data.

## 11.2 MRI data management

At the end of each scan, MRI data will be exported anonymously using the participant's Study ID. Anonymised MR images will be exported in DICOM (Digital Imaging and Communication) format to the shared encrypted research group University of Sheffield Google Drive for primary analysis. Data for primary analysis will be performed with in-house software on an encrypted University of Sheffield computer.

University MR Data is archived on the MRI unit (UniPACS) and transferred to the secure University XNAT network and servers, where it can be stored, archived and processed. These servers have secure access and serve as an imaging informatics software platform. Dr Rea will review the images for incidental findings using this system only and all data will be removed from this system after the review.

## 11.3 Data backup and external use

All data collected or generated through this study will be anonymised with the study ID and backed up on a separate encrypted University of Sheffield Google Drive and password protected, following standard protocols for handling imaging data.

Anonymised MRI data will be electronically transferred to the study investigators Bioxydyn Ltd for independent analysis of the findings. Consortium agreement is established between the two sites. Anonymised data will also be made available for secondary research, educational or commercial purposes by third-party investigators from the private or public sector.

## 11.4 Publications and presentations

All publications and presentations arising from this study are subject to the TRISTAN publication policy. In particular, this requires approval by the TRISTAN publication board prior to submission. Authorship is defined according to ICMJE criteria.

## 11.5 Long-term data storage

Upon completion of the study, imaging data will be fully anonymised and stored in the university-owned Google Drive.  Anonymous data may be made publicly available on zenodo.org in the future, also serving as a long-term repository.

## 11.6 Withdrawal from the study

In the event of a participant withdrawing from the study after one or more scans, unless explicitly requested by the participant any data collected prior to withdrawal will be retained for analysis to protect the integrity of the research data under the general data protection regulation exemption from the right of deletion in research.

### 11.7 End of Study

The end of this study is defined as the date on which the final MRI scan of the final participant is received. We expect this to be a short time after the date of their last visit.

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# 13. Appendix

## 13.1 Serious adverse event reporting form

REPORT OF SERIOUS ADVERSE EVENT (SAE)

A Serious Adverse Event (AE) is a serious untoward medical occurrence or harm, resulting in death, life-threatening illness or injury, or other serious consequences, resulting from research activities. All SAEs should be recorded and reported to the Departmental/School Head of Department and Research Governance Lead **not more than 24 hours** after the study team becomes aware of the event. Following this, a review should be undertaken to assess the conduct and the ongoing suitability of the approved study protocol in light of the serious adverse event, including consideration of the need to report to the NHS REC (see below). The sponsor organisation at Sheffield Teaching Hospitals NHS Trust will be contacted at modhumita.harris@nhs.net. should also be notified within the 24 hour timeframe, and should be consulted on potential actions.

**1. Details of Principal Investigator**

| | |
|---|---|
| Name: | |
| Email: | |
| Department/School: | |

**2. Details of study**

| | |
|---|---|
| Full title of study: | |
| 6 digit project code: | |
| Was the project reviewed by an NHS Research Ethics Committee*?<br>**If so, an NHS SAE form must be submitted* | |

**3. Type of event**

*Please categorise this event, ticking all appropriate options:*

| | | |
|---|---|---|
| Death ☐ | Life threatening ☐ | Hospitalisation or prolonging of existin ☐ hospitalisation |
| Persistent or significant disability or incapacity ☐ | Congenital anomaly or birth defect ☐ | Other ☐ |

**4. Circumstances of event**

| | |
|---|---|
| Date of SAE: | |
| Location: | |
| Describe the circumstances of the event:<br><br>*(Attach copy of detailed report if necessary)* | |
| What is your assessment of the implications, if any, for the safety of study participants and how will these be addressed? Consider both corrective and preventative actions. | |

**5. Declaration**

| | |
|---|---|
| Name of Principal Investigator:<br>*Insert name here*<br>Signature: | Date: *Insert date here* |
| Name of Head of Department/School or Research Governance Lead:<br>*Insert name here*<br>Signature: | Date: *Insert date here* |

**6. Acknowledgement of receipt by the Director of Research, Partnerships & Innovation / Research Ethics & Integrity Manager:**

| | |
|---|---|
| Signed: | |
| Name: | |
| Position: | |
| Date: | |

*Signed original to be sent back to Principal Investigator (or other person submitting report)*
*Copy to be kept for information by Research, Partnerships & Innovation.*

## 13.2 REC reporting form

Report of Serious Adverse Event (For all studies except Clinical Trials of Investigational Medicinal Products)

**The Chief Investigator (CI) should report any SAE that is both related to the research procedures and is unexpected. The report should be emailed to the Research Ethics Committee that gave a favourable opinion of the research within 15 days of the CI becoming aware of the event**

Details of the Chief Investigator

| Question | Answer |
|---|---|
| Name: | Replace text here |
| Address: | Replace text here |
| Telephone: | Replace text here |
| Email: | Replace text here |

Details of the study

| Question | Answer |
|---|---|
| Full title of study: | Replace text here |
| Name of REC: | Replace text here |

| | |
|---|---|
| REC reference number: | Replace text here |
| IRAS ID: | Replace text here |
| Research sponsor: | Replace text here |
| Sponsor's reference for this report:<br>(if applicable) | Replace text here |

Type of event

Please categorise this event, ticking all appropriate options:

Death ☐

Life threatening ☐

Hospitalisation or prolongation of existing hospitalisation ☐

Persistent or significant disability or incapacity ☐

Congenital anomaly or birth defect ☐

Other ☐

Circumstances of event

| Question | Answer |
|---|---|
| Date of SAE: | Replace text here |
| Location: | Replace text here |

| | |
|---|---|
| Describe the circumstances of the event:<br><br>(Attach copy of detailed report if necessary) | Replace text here |
| What is your assessment of the implications, if any, for the safety of study participants and how will these be addressed? | Replace text here |

Declaration

Signature of Chief Investigator:

Print name:

Date of submission:

Acknowledgement of receipt by REC (please insert name):

The [ ] Research Ethics Committee acknowledges receipt of the above.

Signed:

Name:

Position on REC:

Date:

Signed original to be sent back to Chief Investigator (or other person submitting report) Copy to be kept for information by REC.