Biomarker Qualification Letter of Intent (LOI) Content Elements

NOTE TO REQUESTORS: FDA is currently developing its policies for submissions under the 21 Century Cures Act (section 507)[1] and expects to issue guidance to aid in the development of submission based on a decade of reviews, input from public meetings, comments to the docket and collaborative public partnerships. In the interim the Agency has assembled this resource to help requestors. Given the changes to the process as defined in section 507, we expect to see further development of this content over time, with more experience and your input. For additional resources on submission content please see prior Biomarker Qualification Program submissions that we have accepted under section 507 **HERE**. Please also note that certain information contained in submissions will be made publicly available as per section 507, as described in greater detail **HERE**.

Should you have any questions or want to provide feedback on this or other BQP resources, including the content and format of submissions and the transparency provisions under section 507, please contact us at CDER-BiomarkerQualificationProgram@fda.hhs.gov

Administrative Information

1. **Submission Title**:
   Combined use of a pair of imaging safety biomarkers Δk(he) and Δk(bh) to aid identification of investigational drugs that may cause Drug-Drug Interactions

2. **Requesting Organization:**
   Name of Organization
   Bioxydyn Limited on behalf of the IMI-TRISTAN consortium
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3. **Submission Dates:**
   First submission: 15 November 2019
   Revision: 29 May 2020
   Revision: 2 February 2021

Drug Development Need

Numerous clinically relevant drug-drug interactions (DDIs) arise via inhibition or induction of hepatic transporters which mediate uptake and/or biliary excretion of drugs. These may cause potentiated or reduced efficacy that requires drug dose adjustment, and/or increased or reduced toxicity to liver or other tissues. 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, thereby causing myotoxicity [1]. 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 [2]).

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 [3,4]. 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 [4,5]. 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.

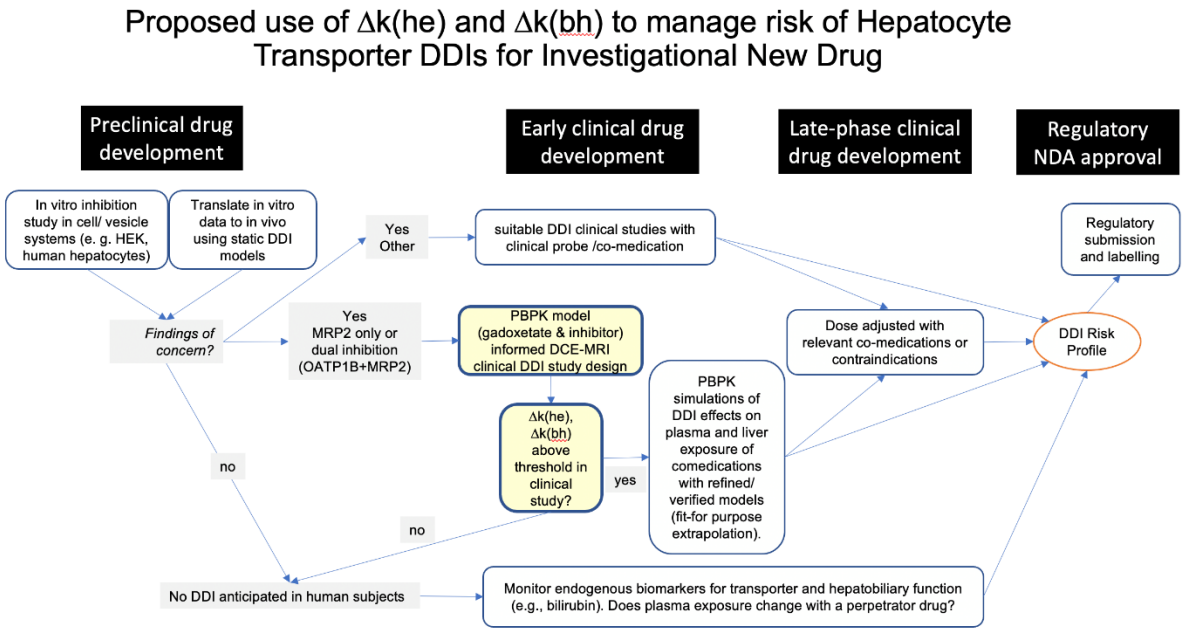
Hence there is a need for noninvasive methods which may be used to determine drug exposure within the tissue of interest *in vivo*. Radiologic imaging is an ideal biomarker modality for this task, as it reports independently from different anatomic compartments. Hepatobiliary scintigraphy can be employed to assess hepatocellular transport function [6], as can PET imaging [7], and, in the past, X-radiography. These methods require the use of ionizing radiation, and require imaging contrast agents or tracers which often are difficult or impossible to access. Use of indocyanine green clearance enables assessment of effects of drugs on liver excretion kinetics [8], but does not provide insight into whether these are caused by effects on hepatic uptake or efflux transporters. Furthermore, indocyanine green is sensitive to liver perfusion impairment and thus may not reflect hepatocyte excretion capacity.

To address these issues we propose the use of a widely available quantitative biomarker modality, dynamic gadoxetate enhanced magnetic resonance imaging (gadoxetate DCE-MRI). The contrast agent gadoxetate is a substrate of liver uptake and biliary efflux transporters. Currently, gadoxetate DCE-MRI is used routinely in the clinic to detect and characterize liver lesions in adults with known or suspected focal liver disease. Previously, we and others have shown in rats that gadoxetate DCE-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 to quantify

the effects on the rate constants of test drugs which inhibit hepatobiliary transporters. We recognize that this biomarker cannot comprehensively address all sources of hepatobiliary drug-drug interactions and that it is limited to transport mechanisms which mediate hepatic uptake and biliary efflux of gadoxetate, namely OATP1B1, OATP1B3, NTCP, MRP2 and MRP3.

This novel liver imaging biomarker pair would be used in drug development to aid assessment of DDI risk. We envisage that the biomarkers will be used following PBPK based analysis of data provided by in vitro drug transporter interaction studies, when DDI risk arising via inhibition of OATPs or MRP2 is suspected. The biomarkers will be used to prioritize IDs for evaluation in an early clinical DDI program. Lack of effect on these biomarkers suggests low risk of DDIs caused by drugs which affect hepatocyte transport function *in vitro* (see Figure 1).

Figure 1. Flowchart showing proposed use of Δk(he) and Δk(bh) to manage risk of Hepatocyte Transporter DDIs for an Investigational Drug



The scientific literature we have cited is listed in attachment 1. The clinical gadoxetate DCE-MRI studies that we will undertake are supported by preclinical studies, which will further demonstrate the robustness and scientific validity of the method and are summarized under "Studies planned to support above considerations".

Biomarker Information and Interpretation

1. **Biomarker name:**

   Δk(he) together with Δk(bh), which quantify investigational drug-induced changes in gadoxetate uptake and efflux transport rate constants for liver tissue, respectively. These are radiographic biomarkers and are proposed to be used as safety (toxicodynamic) biomarkers, i.e. abnormal Δk(he) and/or abnormal Δk(bh) suggest enhanced DDI risk.

2. **Analytical methods:**

Magnetic resonance imaging (MRI). Dynamic contrast-enhanced MRI (DCE-MRI) images are recorded before, and some time after, administration of the investigational drug. Regions-of-interest are manually selected. The biomarkers Δk(bh) and Δk(he) are calculated using an MRI signal analysis and physiologically-based imaging model coded into a computer program. Details are given in section "Analytical considerations".

3. **Measurement units and limit(s) of detection:**

   Transporter rate constants are determined in units of 1/min. Changes are reported as percentage reduction from baseline. Limits of detection and thresholds for relevant inhibition suggesting enhanced risk of DDIs are currently being assessed.

4. **Biomarker interpretation and utility**

   The rate constants k(he) and k(bh) quantify OATP and NTCP-mediated uptake of gadoxetate from extracellular space into hepatocytes, and MRP2-mediated excretion of gadoxetate from hepatocytes into bile, respectively. k(he) also includes potential transport of gadoxetate back into the extracellular space. They are derived via PK model analysis of human gadoxetate DCE-MRI data. In each individual, the biomarkers are measured at baseline and then after administration of the investigational drug. No deviation (strictly, deviation smaller than the threshold) of the biomarkers from baseline value after drug administration indicates no effect of the drug on the rate of hepatic gadoxetate uptake and biliary efflux. Influencing either k(he) and/or k(bh) indicates enhanced potential to cause DDIs by affecting hepatic uptake and/or hepatobiliary excretion of a victim drug which is a substrate of the relevant transporters (OATP1B1, OATP1B3, NTCP, MRP2 and MRP3). We envisage that determination of the changes in transport rate constants Δk(he) and Δk(bh) will be undertaken particularly in clinical dose finding trials to inform detection of possible transporter mediated DDIs which previously have been evaluated by PBPK model based analysis of in vitro transporter inhibition data.

   As depicted in Figure 1, the DDI potential of a new drug will initially be evaluated in pre-clinical in vitro and in vivo tests and subsequently also in humans applying PBPK approaches fed with data from OATP1B inhibition studies and hepatobiliary function impairment examinations. These data inform the DDI risk profile.

   In case of a detected DDI risk, utilizing k(he) and k(bh) to further refine PBPK modelling of the drug of interest, will help to improve the mechanistic understanding of the DDI risk (hepatic uptake or efflux mediated) and also will help to resolve potential drug tolerability issues by appropriate adaptation of the drug dose.

   This biomarker pair will be particularly useful to understand the impact of drugs being substrate of OATP1B1/1B3, NTCP, MRP3 or MRP2 that have different concentrations between plasma and hepatocytes or have a pharmacologic target inside hepatocytes and their pharmacologic effect will thus be impacted by altered uptake or excretion to or from hepatocytes.

Context of Use Statement (500 characters)

A safety (acute lack-of-harm) biomarker, employed in the development of investigational drugs (IDs) which are thought to carry an enhanced DDI risk because prior animal or in vitro human cell studies have indicated that the ID is a hepatic transporter inhibitor or inducer. Δk(he) and Δk(bh) would be used in early phase clinical drug development. An ID whose effect on either Δk(he) or Δk(bh) is not below-threshold would be prioritized for an early program of clinical DDI investigations.

BEST biomarker category: Safety.

Analytical Considerations

The rate constants k(he) and k(bh) for gadoxetate uptake from the liver's extracellular space into hepatocytes, and excretion from hepatocytes into bile, respectively, are determined by dynamic gadoxetate DCE-MRI as follows.

Our general approach is that each subject is scanned on two occasions each with identical MRI protocols, once before and once after administration of the investigational drug. The time between the two scan sessions is long enough for gadoxetate to clear from the first session and long enough for the investigational drug to reach peak plasma concentration, but otherwise should be as short as logistically possible (typically 2-7 days). Our aim is to achieve k(he) and k(bh) repeatability that is small in comparison with clinically significant changes in k(he) and k(bh), so that results can be interpreted at the level of the single subject and do not need significance tests of groupwise changes.

Image acquisition: Acquisitions can be performed on 1.5T or 3T MRI scanners from any vendor, provided the required sequences are available as products. T_1- and T_2-weighted series of images are acquired for anatomical reference. A standard 3D spoiled gradient echo pulse sequence is applied with variable flip angles for measurement of precontrast longitudinal relaxation rate $R_{1,0}$. A B_1-mapping protocol (normally provided by the manufacturer) is applied in order to measure the flip angles across the 3D volume. Subsequently, the variable flip angle sequence is applied dynamically with a fixed flip angle, acquiring at least 20 3D volumes per minute over at least 30 minutes. Gadoxetate is injected by power injector at a quarter of a standard clinical dose (0.025mL/kg bodyweight) and a rate of 1mL/s, 30s after start of the acquisition. The contrast is flushed with 20mL of saline at the same rate.

Image analysis: Regions of interest (ROIs) are defined semi-automatically covering the entire liver (excluding vessels), and in the abdominal aorta and in the portal vein. Detailed SOPs for ROI placement in liver and vessels are provided along with test data sets and reference values to aid in the training of new operators. Flip angles α are measured for each pixel from the B -maps and signal intensity vs measured flip angle curves S(α) are derived for the liver. Signal intensity vs time curves S(t) are derived for all liver pixels and vessels.

In a first step the pre-gadoxetate liver $R_{1,0}$ for each pixel is determined by fitting S(α) using the standard signal model for a spoiled gradient echo MRI sequence in the steady state:

[Eq. 1]

$$S(\alpha) = S_0 \sin(\alpha) \frac{1 - e^{-TR \cdot R_{1,0}}}{1 - \cos(\alpha)\, e^{-TR \cdot R_{1,0}}}$$

Here TR is the repetition time, a known sequence parameter. The $R_{1,0}$ values for abdominal aorta and portal vein are derived from a literature formula for $R_{1,0}$ (1/s) in arterial and venous blood, including a correction for patient-specific hematocrit at 3T [9].

[Eq. 2]

$$R_{1,0} = A\, Hct + B$$

The values for A and B are given in the table 1:

Table 1

	3T - arterial	3T - venous	1.5T - arterial	1.5T - venous
A (1/s)	0.52	0.83	0	0
B (1/s)	0.38	0.28	0.67	0.67

In a second step the signal-time curves for liver, aorta and portal vein are converted to $\Delta R_1(t)$ curves using the same signal model:

[Eq. 3]

$$\Delta R_1(t) = -\frac{1}{TR} ln \frac{1-E(t)}{1-\cos(\alpha)E(t)} - R_{1,0} \quad \text{with} \quad E(t) = \frac{1-e^{-TR \cdot R_{1,0}}}{1-\cos(\alpha)e^{-TR \cdot R_{1,0}}} \frac{S(t)}{S_0}$$

This step produces three curves $\Delta R_{1L}(t)$, $\Delta R_{1A}(t)$ and $\Delta R_{1P}(t)$ for liver, aorta and portal vein, respectively.

In a third step, a dual-inlet two-compartment PK model is fitted, allowing for different relaxivities in each compartment:

[Eq. 4]

$$\Delta R_{1,L}(t) = \left(e^{-t/T_e} + \kappa\, e^{-t/T_h} * e^{-t/T_e}\right) * \left(F_A \Delta R_{1,A}(t - \tau_A) + F_V \Delta R_{1,V}(t)\right) / (1 - Hct))$$

Here Hct is the patient-specific Hematocrit measured in a blood test. The free parameters fitted from the model are T_e, T_h, κ, F_A, F_V, τ_A. Model fitting is performed by Levenberg-Marquardt least squares optimization. From these parameters, the rate constants k_{he} and k_{bh} are derived in the following steps:

$$E = \kappa T_e \frac{r_{1p}}{r_{1h}}; \quad k_{he} = \frac{E}{1-E}(F_A + F_V); \quad v_e = T_e(F_A + F_V + k_{he}); \qquad k_{bh} = \frac{1 - v_e - Hct * v_b}{T_h}$$

The new parameters in these three equations are treated as known constants and fixed to reference values [10,11]:

- vb = 0.17 (liver blood volume fraction)
- r1h = 14.6 $s^{-1}mM^{-1}$ at 1.5T and 9.8 $s^{-1}mM^{-1}$ at 3T (hepatocyte relaxivity of gadoxetate)
- r1b = 8.1 $s^{-1}mM^{-1}$ at 1.5T and 6.4 $s^{-1}mM^{-1}$ at 3T (blood relaxivity of gadoxetate)

Deployment of the assay: Two approaches are provided, a maximally centralized and a maximally decentralized model.

In the centralized model, Δk(he) & Δk(bh) biomarker assays are provided as a service by a Clinical Research Organization, Bioxydyn Ltd (Manchester UK). In compliance with ICH Good Clinical Practice, and in particular the ICH GCP rules on the validation of computerized systems, Bioxydyn performs site qualification, QA/QC and analysis using its VoxelFlow software.

In the decentralized model, protocols, standard operating procedures, training materials, benchmarking data and software for acquisition and analysis is provided to individual laboratories to allow them to develop their own Δk(he) & Δk(bh) imaging biomarker assay. The materials will be made publicly available via version-controlled public website and maintained by the Laboratory of Medical Imaging Physics at the University of Sheffield, UK. Software will be provided as an open source plugin for *Weasel*, a new software tool for prototyping and deploying quantitative medical imaging analysis developed jointly by TRISTAN consortium and the UK renal imaging network. *Weasel* is being developed according to good software development practice with proper attention to version control, transparency, sustainability and documentation for users and developers. *Weasel* will be made

available through an Apache 2.0 license to impose minimal restrictions on future use and redistribution. The *Weasel* implementation of the analysis will be validated against an implementation in PMI 0.4, an open source software tool for medical imaging analysis that has been used in over 100 published papers including the preclinical validation studies performed by the TRISTAN consortium.

Site qualification: Initially the biomarker is to be measured on commercially-available, 510(k)-cleared whole-body MRI scanners sold for example by GEHC, Philips, or Siemens, and operating at 1.5T or 3T. Since different makes and models of scanners exist in different hospitals, and future scanner upgrades, makes and models have the potential to affect the measurement of the biomarker, a specific site qualification procedure and QA/QC procedures will be employed for all scanners measuring this biomarker.

Quality assurance procedures include

1. Verification that the scanner provides suitable acquisition capabilities
2. Training of staff in the MRI acquisition laboratory
3. Verification that R_1 can be determined over a suitable range, with suitably low bias and variance at baseline, and dynamically by performing MRI experiments on a suitable reference object (phantom).
4. Training of staff in the analysis laboratory
5. Verification at the analysis laboratory that scans have been performed correctly and acquired with suitably low levels of artefact and noise.
6. Verification at the analysis laboratory that all MR biomarkers and other derived parameters are within the expected range with suitably low variance.

Clinical considerations

General considerations on qualified use

- For use in clinical trials for drugs potentially possessing a DDI risk in study subjects
- To be used in conjunction with clinical biomarkers assessing DDI risk profile of the test drug and appropriate PBPK modelling
- To be used to assess the mechanism of hepatobiliary excretion impairment
- To be used to inform test drug dose adaptation

Population consideration

- Participant population are adults aged 18 or above
- Eligible for early-stage clinical studies of the investigational drug
- No contra-indications to gadoxetate or MRI

Data acquisition considerations

- Modality: Magnetic Resonance Imaging (MRI) at 1.5T or 3T
- Widely available on most MR manufacturers and models
- Routine MR safety screening applies

Biomarker interpretation

An investigational drug whose effect on both Δk(he) or Δk(bh) is below-threshold would not be considered to be of significant risk of DDIs associated with MRP2- or dual (OATP1B+MRP2)-inhibition, and would not require dose-adjustment or contra-indications to avoid such putative DDIs.

Studies planned to support above considerations

Travelling volunteer study. A travelling volunteer study was performed to determine repeatability and reproducibility of T_1 maps with the TRISTAN protocol and provide benchmarks for future site qualification and QA. Eight healthy volunteers with no known liver disease or MRI contraindications were scanned twice each on six MRI scanners from three vendors at two clinically used field strengths of 1.5T and 3T. The maximum time period between the first and last scans amongst all volunteers was 9 months. Volunteers were instructed to abstain from alcohol intake for at least 72 hours prior to each scan and avoid high cholesterol foods on the day of the scan. A NIST system phantom was also subject to the same MRI protocol and scans. Analysis of the data is ongoing.

> *Rationale for the study: k(he) & k(bh) are calculated from liver T_1 and T_1 change; a robust assay will control the accuracy, repeatability and reproducibility of these underlying parameters; the data from the study will help us design QA/QC procedures and thresholds for use in commonly-used makes and models of MRI scanner.*

Rifampicin volunteer study. Participants will have a gadoxetate DCE-MRI at baseline. Subsequent treatment with a single dose of rifampicin will be given followed by gadoxetate DCE-MRI two hours post-dose. Δk(he) & Δk(bh) will be measured with the TRISTAN protocol and provide benchmarks for effect size and between-subject variability. Progress with this study has been paused due to the COVID-19 pandemic.

> *Rationale for the study: Rifampicin is implicated in DDIs and believed to affect Δk(he) & Δk(bh); the study will address reproducibility of the assay; the reproducibility and effect size will help us set threshold above which reduction in Δk(he) & Δk(bh) would be of concern.*

Five-day study of two further drugs in volunteers. Participants will have a gadoxetate DCE-MRI at baseline. Subsequently there will be two treatment arms involving treatment for up to 5 days with different marketed drugs known to inhibit OATP1B1, BSEP and MRP2 function and cause clinically relevant transporter DDIs and infrequent human drug-induced liver injury. These data will be used to follow inhibition and/or up-regulation of transporter activity in response to challenge, and to develop and refine the human PBPK model. Progress with this study has been paused due to the COVID-19 pandemic.

> *Rationale for the study: to further understand relevant effect size and limits of detection, help us set threshold above which reduction in Δk(he) & Δk(bh) would be of concern.*

Cholestatic pruritis study. Investigation of the effect of a clinical dose of rifampicin, a known

inhibitor of BSEP, MRP2 and OATP, on gadoxetate disposition in volunteers and in patients with cholestasis and pruritus. This study will provide insight into whether gadoxetate DCE-MRI is suitable for evaluation of drug induced effects in patients with pre-existing cholestasis and will investigate the link between alterations in hepatobiliary transporter function and changes in plasma bile acids and pruritus symptoms, in order to better understand their inter-relationship and to develop and refine the human PBPK model. Rifampicin is indicated clinically for treatment of cholestatic pruritis, however not all patients respond. This study provides a particularly strong test of the biomarker, as multiple dosing of rifampicin may induce complex induction of multiple proteins. Progress with this study has been paused due to the COVID-19 pandemic.

Rationale for the study: Rifampicin is implicated in DDIs and believed to affect Δk(he) & Δk(bh); the study will address reproducibility of the assay; the reproducibility of the effect will help us set threshold above which reduction in Δk(he) & Δk(bh) would be of concern.

Supporting Information

Literature data indicate that gadoxetate is an *in vitro* substrate of multiple human hepatobiliary uptake (OATP1A1, OATP1B1, OATP1B3, NTCP) and efflux (MRP2, MRP3) transporters [12,13,14]. Our in vitro studies demonstrated that gadoxetate is not a BSEP substrate and exhibited concentration-saturable uptake into rat hepatocytes [manuscript in preparation].

Proof of principle of the gadoxetate DCE-MRI method has been provided by studies undertaken previously in rats and in human. Studies that we have undertaken in rats have demonstrated in perturbation of hepatic clearance of gadoxetate by compounds which include rifampicin [15], an investigational chemokine antagonist [13] and estradiol-17β D-glucuronide [16]. These drugs inhibit either Oatp1b2 and/or Mrp2 in vitro [13,15,16]. These findings indicate that gadoxetate liver DCE-MRI can be a sensitive probe for perturbed function of Oatp 1b and Mrp2 transporters. In addition, the use of physiologically-based imaging models for estimating uptake and efflux transporter rate constants from gadoxetate DCE-MRI data in human and rat has been reported in the literature, by us and by other investigators [13,15,16,17,18,19,20].

Published examples of substances which exhibiting non-zero Δk(he) & Δk(bh)		
compound	observation	reference
rifampicin	In rats treated in vivo, both the uptake and the washout of gadoxetate was significantly slowed	[21] [22]
estradiol-17β D-glucuronide	In rats treated in vivo, the washout of gadoxetate was significantly slowed, interpreted as impairment of biliary excretion.	[16]
Investigational chemokine antagonist 1-(4-chloro-3-trifluoromethyl-benzyl)-5-hydroxy-1-H-indole-2-carboxylic acid, a known inhibitor	In rats in vivo, the substance reduced both the MRI-determined rate of uptake of gadoxetate into the hepatocyte and also the Michaelis–Menten constant characterizing its excretion into bile, while the Michaelis–Menten constant for	[13]

of ATP-dependent transporter activity of rat Bsep and Mrp2	biliary efflux increased. These effects were dose dependent.	

The work that we are undertaking to validate imaging biomarkers of liver transporter function are being undertaken as a part of the "TRISTAN" project. TRISTAN is a public-private partnership funded via the EU framework program "Innovative Medicines Initiative-2". The goal of TRISTAN is to initiate introduction of valid imaging biomarkers to help avoid, mitigate and manage drug-induced harms. Further information on the entire TRISTAN work program is available at the project website: www.imi-tristan.eu. Within this overall goal, the goal of one TRISTAN workstream is to establish imaging biomarkers to help avoid, mitigate and manage drug-induced harms to the liver; and in particular detection of drug-drug interactions caused by inhibition, activation or malfunction of the hepatocyte transporters Oatp/OATP, Mrp2/MRP2 and Bsep/BSEP. Foremost, inhibition, activation or malfunction of Oatp/OATP, Mrp2/MRP2 is determined by employing dynamic gadoxetate enhanced MRI. The work includes extensive supporting preclinical studies in vitro and also in vivo in rats. Plans are summarized in attachment 2.

Previous Qualification Interactions and Other Approvals (if applicable)

None

Attachments

- Attachment 1: Publications cited in the LOI
- Attachment 2: Planned studies
  - Table s2 summarizes literature data on human hepatic transporter inhibition effects of the six tests drugs we intend to use on our gadoxetate DCE-MRI studies.
  - Table s3 summarizes the studies that we plan to undertake.
  - Figure s2 illustrates the expected process for verification and qualification of the preclinical and clinical liver imaging biomarker for evaluation of DDI risk mediated via hepatic transporters.
  - Figure s3 illustrates the proposed use of the gadoxetate DCE-MRI biomarker to evaluate hepatic transporter (OATP1B/MRP2 perpetrator) mediated DDI risk for a novel drug candidate in human.

Additional Information & Submission Information:

This LoI is submitted by the TRISTAN consortium, a public-private partnership developing imaging biomarkers pertinent to drug safety under the EU "Innovative Medicines Initiative" www.imi-tristan.eu. The Context of Use in this document sits within a larger framework of possible biomarkers and contexts of use, some of which are also investigated within TRISTAN

Other contexts of use for the same or closely similar gadoxetate-based liver imaging biomarkers

- Predictive biomarkers for use in patient selection or treatment selection
- Biomarkers suggesting enhanced risk of drug-induced liver injury

Attachment 1: Publications cited in the LOI

1. Shitara Y, Hirano M, Sato H, Sugiyama Y. Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. J Pharmacol Exp Ther. 2004 Oct;311(1):228-36. doi: 10.1124/jpet.104.068536. Epub 2004 Jun 11. PMID: 15194707

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Attachment 2: Further information on planned studies

- Table s2 summarizes literature data on human hepatic transporter inhibition effects of six tests drugs explored in our gadoxetate DCE-MRI studies.
- Table s3 summarizes the studies that are underway or in the protocol development stage
- Figure s2 illustrates the expected process for verification and qualification of the preclinical and clinical liver imaging biomarker for evaluation of DDI risk mediated via hepatic transporters.

Table s2. Selected test drugs being evaluated in rats and their human hepatic transporter IC50 or Ki (µM) data reported in literature using in vitro assays [a]

Compound	OATP1B1	OATP1B3	NTCP	MRP2	BSEP
Rifampicin	0.22 (1.24; 120)	0.11 (1.4; 190)	277	7.9 (33.8; 144)	10.5 (34; 204)
Cyclosporine A	0.014 [b] (0.28; 3.5)	0.032 [b] (0.50; 3.1)	0.37 (2.1; 7.6)	2.69 (6.83; 45.3)	0.27 (2.11; 100)
Bosentan [‡]	5 (-; 8.2)	5.2 (-; 9.9)	18 (29.2; 50.9)	NI @ 100µM	12 (26.6; 100)
Ketoconazole	1.8 (17.9; 107.7)	3.9 (16.3; 20)	202 (233; 264)	NI @ 20µM	2.3 (4.65; 65.4)
Asunaprevir	0.3 (-; 0.795)	0.65 (-; 3)	ND	4	2.2
Pioglitazone	5.09 (11.05; 39.6)	3.41 (-; 6.55)	4.04	NI @ 133µM	0.02 (0.3; 100)

[a] Lowest value found in literature included in table. Values in parentheses represent median and highest value found.

[b] Pre-incubation effects on IC50 reported

[‡] Stimulation of MRP2 transport reported by bosentan

- Only two values reported, no median indicated

NI – No inhibition at concentration indicated; ND – No data found

Table s3. Summary of completed, ongoing and planned gadoxetate studies being undertaken within TRISTAN.

Study name	Subjects	Endpoint
In vitro kinetic profiling of hepatocyte transporters (Project Task 2.01)	n.a. (cell assays)	In vitro "ground truth" kinetic profiles of hepatocyte transporters is determined
Stability of MRI longitudinal relaxation rate	n.a. (phantom	Deviation of R_1 measurements

R_1 in 18 clinical and preclinical MRI devices at field strength 1.5T – 11.7T (Project Task 2.02)	study)	determined for different MR scanners <10%
Investigation of changes in kinetics of gadoxetate after uptake into hepatocytes in preclinical MRI devices at field strength 4.7T, 7T (Project Task 2.02)	6 Crl:WI (Han) rats / imaging centre and MR field strength	change upon cell uptake of gadoxetate kinetics determined for each device
Reproducibility and repeatability of gadoxetate kinetics in rats in preclinical MRI devices at field strength 4.7T, 7T (Project Task 2.02)	6 Crl:WI (Han) rats / imaging centre and MR field strength	Deviation of rate constants determined in vivo <15%
Reproducibility and repeatability of R_1 measurements in healthy volunteers using the DCE-MRI image acquisition protocol (but *without* contrast agent) at different vendors' MR scanners at field strength 1.5T and 3T (Project Task 2.02)	5 healthy volunteers travelling between 2 imaging centres	Deviation of R_1 determined in vivo <15%
Reproducibility and repeatability of gadoxetate enhanced DCE-MRI in rats at 4 preclinical MRI devices at field strength 4.7T, 7T (Project Task 2.06)	6 Crl:WI (Han) rats / imaging centre and MR field strength	Deviation in determined baseline transporter activity <20%
Investigation of the effects of single doses of 6 selected test drugs in the established imaging biomarker assay at field strength 4.7T, 7T in 4 centres (Project Task 2.06)	36 Crl:WI (Han) rats / imaging centre and MR field strength	Drug effects observed in vitro and ex vivo can be reproduced in vivo between imaging sites with a deviation of <20%
Investigation of the effects of repeated daily dosing of 2 selected test drugs for up to 5 days of rats in the established imaging biomarker assay at field strength 4.7T, 7T	12 Crl:WI (Han) rats / imaging centre and MR field strength	Drug effects observed ex vivo can be reproduced in vivo between imaging sites with a deviation of <20%
Experimental study, with adaptive clinical trial design to quantify effects on gadoxetate enhanced DCE-MRI after administration of a single dose of rifampicin to healthy volunteers at different vendors MR scanners at 1.5T and 3T (Project Task 2.13)	10 healthy volunteers	Suitability of a clinical DCE-MRI protocol is verified
Investigation of effects of a single dose of 2 test drugs on gadoxetate DCE-MRI in human healthy volunteers (Project Task 2.06)	20 healthy volunteers	DCE-MRI imaging biomarker method developed in rats is directly translatable to humans; the effect of the test drug by DCE-MRI is significant
Intervention study to quantify effects on gadoxetate enhanced DCE-MRI after administration of a single dose of rifampicin in healthy volunteers and patients with cholestasis and pruritus (Project Task 2.07)	8 healthy volunteers and 8 cholestasis and pruritus patients imaged in 2 centres	Change in transporter activity induced by rifampicin matches with adjusted rat data; the effect of rifampicin by DCE-MRI is significant

R_1: magnetic resonance longitudinal relaxation rate of protons in ^1H isotope in water

Figure s2. Flowchart illustrating the expected process for verification and qualification of the preclinical and clinical liver imaging biomarker for evaluation of DDI risk mediated via hepatic transporters