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PUBLISHABLE SUMMARY

This report concerns ‘toxicology-associated targets’, which are biomolecules that can initiate or otherwise mediate a toxic response when they interact with a xenobiotic. Targets of interest are those with roles in hepatotoxicity, cardiotoxicity and drug disposition. Various target classes are discussed, along with the evidence supporting their inclusion. Finally, there is a discussion of issues encountered in compiling the set of toxicology-associated targets and ways to refine and progress this work going forward.

OBJECTIVES

Among the objectives of HeCaToS Work Package 9 are:

- Provide centralized access to xenobiotic binding, pharmacology and ADMET data;
- To identify knowledge-gaps and procure any available data to fill these gaps.

This report involves these objectives, in that it would be clearly desirable for the project to have easy access to as much data as possible for key targets of relevance to toxicology. This will facilitate the work of modellers in various work packages and will in turn enable the hierarchical modelling strategy envisioned for the HeCaToS system.

The ChEMBL database (<https://www.ebi.ac.uk/chembl>) [1], which will be a key component of the HeCaToS data infrastructure, already contains much data useful to toxicology modelling. However, the utility of that data could in some cases be improved by further curation, enhanced indexing and cross-database integration and linkage. Further, valuable data might exist in the literature that is not yet included in the ChEMBL, and extraction and uploading of such data would be desirable.

However, both of these activities can be time-consuming and/or costly and prioritisation of efforts is thus required. By compiling a reference set of toxicology-associated targets and focussing our efforts on these targets, we should be able to deliver maximum value to the project partners and the public.

INTRODUCTION

The aim of this report is to present a reference set of toxicology-associated targets. The concept of ‘target’ in pharmacology and toxicology can be used to mean different things; for example, in attempts to model drug-induced liver injury (DILI), the organ itself is sometimes considered as the target of a chemical [2]. For a variety of reasons, including the multiple mechanisms of hepatotoxicity and many possible confounding factors [3, 4], this whole-organ approach has had limited success.

In this report, we will generally consider a ‘target’ to be a specific biomolecule, *i.e.* a protein or protein complex of defined stoichiometry with which xenobiotics might interact to produce an adverse response; these are often referred to as anti-targets or off-targets in order to distinguish them from therapeutic targets [5, 6]. Note that a biomolecule may be considered a therapeutic target or anti-target depending on context; it can be beneficial to engage a target in a particular disease state, but harmful in another. A number of databases of toxicity-associated targets already exist [7, 8], which, while not exhaustive, do contain useful information.

Restricting the definition to molecular-level targets is appropriate for several reasons. For example, such an interaction could be the molecular initiating event (MIE) of an adverse outcome pathway (AOP) [9], a concept increasingly being used to formalise thinking about adverse responses to xenobiotics. In addition, such molecular-level data could be used to build computational models [10]

underpinning a multi-scale approach to toxicity prediction [11, 12], an idea and modelling strategy which itself meshes well with the AOP concept [13].

For certain classes of targets such as GPCRs, nuclear receptors or ligand-gated ion channels, there can be multiple modes of engagement, such as agonism, antagonism and perhaps inverse agonism [14]. Toxic effects might be caused by one or other mode of engagement and, when annotating anti-targets with associated toxicities, the mode should be specified whenever possible. However, this information is not always known, such as where the only data is from a binding assay (yielding *e.g.* a K_d end point), and this can complicate the interpretation and use of the data considerably.

Although MIEs often involve binding to the receptors and enzymes on which we shall focus here, they may also involve less specific events such as protein alkylation [15]. In addition, a toxic insult, like a therapeutic effect, may require engagement of more than one target and it might be the activity *profile* that is important [16, 17]. In other words, at least in some cases, engagement of a combination of individually innocuous targets might be required to induce a toxic effect. This would make associating targets with adverse drug reactions (ADR) more difficult and also complicate modelling strategies. However, there is also evidence that side effects tend to be mediated by interaction of drugs with individual proteins [18].

Given the strategic aims of the HeCaToS project, the focus will be on anti-targets of relevance to hepatotoxicity and cardiovascular toxicity and on the machinery controlling drug disposition. In compiling the report, the decision was made to include only those targets that have some level of validation available. There have been various publications describing attempts to link molecular targets to drug adverse events or side effects statistically [18-21], but, while these *potential* novel anti-targets are interesting, they do not tend to come with any independent verification or with a clear mechanistic rationale. Such targets will thus be kept in mind for future data-gathering and/or modelling purposes, but will not be considered further at this time.

A pragmatic method of identifying anti-targets with at least some level of validation is to take those that are used for *in vitro* profiling in a drug discovery setting [6, 22], where they are either published by pharmaceutical companies [23, 24] or appear in the assay catalogues of contract research organisations (CRO) used by these companies [25, 26]. This should give a fairly conservative set of anti-targets, which could then perhaps be augmented with newer or more speculative examples from the literature.

One issue with this approach is that the targets in these lists are not always annotated with the organ or tissue in which they exert a toxic effect, so it might not be apparent which contribute to cardiovascular or hepatic toxicity specifically. This could potentially be addressed to some extent by automatic annotation using a tissue expression database [27, 28], although analysis of the literature for each target individually would be required for confidence in the conclusions. Furthermore, the reasons for inclusion (*i.e.* the weight of evidence, a mechanistic rationale *etc.*) are not always given and, again, this could only really be addressed by consulting the literature.

The effects of xenobiotics on mitochondria are very important for understanding both hepatotoxicity [29, 30] and cardiotoxicity [31, 32] and modelling of mitochondrial toxicity is considered an important part of EMBL-EBI's contribution to HeCaToS WP1. Mitochondrial biochemistry is well described by reaction/pathway databases such as Reactome [33]; this is valuable as pathways can be used as an organising framework for modelling activities.

Thinking in terms of pathways and systems, in mitochondria and beyond, provides deeper insight into mechanisms of toxicity and is clearly compatible with the AOP and multi-scale concepts discussed above. Data for mitochondrial targets could also be of use for the dynamic modelling [34] strategies being pursued in HeCaToS WP2. This in turn could inform WP1 activities, for example by

identifying those components of pathways that are most likely to disrupt proper cell functioning if inhibited and which should therefore be prioritised for further investigation.

The xenobiotic metabolising enzymes (XME) and transporters involved in drug disposition are another special class of anti-target [35]. Although they can be involved in direct toxicity [4], interactions with these entities are most likely to cause problems in indirect ways, such as by generating reactive metabolites or by altering the distribution or metabolism of co-administered species and causing so-called drug-drug interactions (DDI) [36]. These entities are thus of interest for toxicity prediction, even where they do not quite fit the definition of anti-target given above.

When considering the potential for DDIs, organs other than those that are the focus of HeCaToS must also be considered. XMEs and transporters are present in many tissues such as the GI tract and the kidneys [37, 38], and inhibition or induction of any of these by a drug could have an impact on the concentration of other species.

Although the focus of this report will be on molecular targets, this does not mean that data gathering should be restricted to such assays. Data for assays conducted on other levels will also be valuable for multi-scale modelling, both for validating bottom-up models and for enabling complementary ‘middle-out’ approaches [39]. An example would be the use of cell-based assays for measuring drug-induced mitochondrial dysfunction [40], or high-content screening assays for DILI [41]. Even compendia of hepatotoxic drugs [42], while not necessarily attractive for modelling purposes, could be useful for testing hypothesis generated from lower level models.

Although the focus is on toxicants that exert their effect *via* molecular targets, there are some of interest to this project that do not exert their effect by direct interactions with biomolecules, but rather *via* intrinsic chemical properties: examples would be mitochondrial uncouplers [43] and compounds capable of redox-cycling [44].

1. RESULTS

1.1. Cardiovascular Targets

In some cases, as with hERG and arrhythmias, the link between an anti-target and the associated toxicity is relatively well (although certainly not *fully*) understood [45]. Further, in this case, the anti-target is clearly localised to cardiac tissue. In some cases, however, the linkage is less direct and possibly multifactorial [46, 47]. An example would be the effects of NSAIDs, where COX2 inhibition reduces prostaglandin synthesis, leading to vasoconstriction and, *via* effects on the kidneys, to water retention; together, these effects can lead to heart failure in vulnerable populations [47]. Such complexities must always be borne in mind when attempting to link data for molecular anti-targets to organ-level (or higher) effects.

A recent perspective on the use of *in vitro* profiling in the drug discovery process gave a list of targets recommended by representatives of multiple pharmaceutical companies as a core panel for early assessment of possible safety-related liabilities [24]. These were annotated with the organ(s) primarily affected and a list of pathological effects (with references), broken down by interaction type. Of the 44 listed, 30 have the cardiovascular system (CVS) as one of the organs affected: these are shown in Table 1. The effects are broken down by agonism/activation vs. antagonism/inhibition, the importance of which was noted above, and references are provided for each target. Both because of the provenance and the level of annotation, this would seem to be an excellent starting set of cardiovascular targets.

Note that, in some cases, the effects listed do not seem to include any obviously cardiovascular in nature; for examples, see the μ -opioid receptor and the serotonin transporter. However, a brief inspection of the literature suggests there is evidence of μ -opioid receptors affecting the CVS [48]. Further, given there are several serotonin receptors included, that the associated transporter should be an anti-target would certainly seem plausible. These cases illustrate how further research and/or mechanistic thinking might be necessary to understand the rationale for the inclusion of anti-targets, even in a list as well-annotated as this one.

It is explicitly stated that this consensus list comprises a *minimal* set of assays, and that the companies involved all screen other targets in addition. For example, an earlier, but still widely cited, review of the same topic included a list of cardiovascular targets screened during the discovery phase at Novartis [23]; these are presented in Table 2, with those also appearing in the consensus list in Table 1 highlighted.

It is interesting that many of the extra targets belong to families already seen (*e.g.* the adenosine or adrenergic receptors), or are part of common pathways or systems (*e.g.* the ATP-sensitive K⁺ channel has a role in the cardiac action potential – see below). Pursuing these types of relationship in the literature would be a rational way of expanding the list of anti-targets, if that should prove desirable; this topic is discussed at more length below.

Note that, although some information on possible ADRs is provided in this second list, it is not split out by interaction type (*e.g.* agonism vs. antagonism). This is information that would be vital in linking target interactions to phenotypes, and thus might require further literature research.

Table 1: Cardiovascular targets taken from Table 1 in reference [24]. Note that there are references for each entry in the original.

Target	Gene	Organ(s)	Effects Agonism or activation	Effects Antagonism or inhibition
Adenosine receptor A _{2A}	ADORA2A	CVS, CNS	Coronary vasodilation; decrease in BP and reflex; increase in HR; decrease in platelet aggregation and leukocyte activation; decrease in locomotor activity; sleep induction	Potential for stimulation of platelet aggregation; increase in BP; nervousness (tremors, agitation); arousal; insomnia
α_{1A} -adrenergic receptor	ADRA1A	CVS, GI, CNS	Smooth muscle contraction; increase in BP; cardiac positive inotropy; potential for arrhythmia; mydriasis; decrease in insulin release	Decrease in smooth muscle tone; orthostatic hypotension and increase in HR; dizziness; impact on various aspects of sexual function
α_{2A} -adrenergic receptor	ADRA2A	CVS, CNS	Decrease in noradrenaline release and sympathetic neurotransmission; decrease in BP; decrease in HR; mydriasis; sedation	Increase in GI motility; increase in insulin secretion
β_1 -adrenergic receptor	ADRB1	CVS, GI	Increase in HR; increase in cardiac contractility; electrolyte disturbances; increase in renin release; relaxation of colon and oesophagus; lipolysis	Decrease in BP; decrease in HR; decrease in CO
β_2 -adrenergic receptor	ADRB2	Pulmonary, CVS	Increase in HR; bronchodilation; peripheral vasodilation and skeletal muscle tremor; increase in glycogenolysis and glucagon release	Decrease in BP
Dopamine receptor D ₁	DRD1	CVS, CNS	Vascular relaxation; decrease in BP; headaches; dizziness; nausea; natriuresis; abuse potential	Dyskinesia; parkinsonian symptoms (tremors); anti-emetic effects; depression; anxiety; suicidal intent
Dopamine receptor D ₂	DRD2	CVS, CNS, endocrine	Decrease in HR; syncope; hallucinations; confusion; drowsiness; increase in sodium excretion; emesis; decrease in pituitary hormone secretions	Orthostatic hypotension; drowsiness; increase in GI motility
Endothelin receptor A	EDNRA	CVS, development	Increase in BP; aldosterone secretion; osteoblast proliferation	Teratogenicity
Histamine H ₁ receptor	HRH1	CVS, immune	Decrease in BP; allergic responses of flare, flush and wheal; bronchoconstriction	Sedation; decrease in allergic responses; increase in body weight
Histamine H ₂ receptor	HRH2	GI, CVS	Increase in gastric acid secretion; emesis; positive inotropy	decrease in gastric acid secretion
δ -type opioid receptor	OPRD1	CNS, CVS	Analgesia; dysphoria; psychomimetic effects; cardiovascular effects; convulsion	increase in BP; increase in cardiac contractility
κ -type opioid receptor	OPRK1	GI, CNS, CVS	decrease in GI motility; increase in urinary output; sedation and dysphoria; confusion; dizziness; decrease in locomotion; tachycardia	Insufficient information
μ -type opioid receptor	OPRM1	CNS, GI, CVS	Sedation; decrease in GI motility; pupil constriction; abuse liability; respiratory depression; miosis; hypothermia	increase in GI motility; dyspepsia; flatulence
Muscarinic acetylcholine receptor M ₁	CHRM1	CNS, GI, CVS	Proconvulsant; increase in gastric acid secretion; hypertension; tachycardia; hyperthermia	decrease in cognitive function; decrease in gastric acid secretion; blurred vision
Muscarinic acetylcholine receptor M ₂	CHRM2	CVS	decrease in HR; reflex; increase in BP; negative chronotropy and inotropy; decrease in cardiac conduction (PR interval); decrease in cardiac action potential duration	Tachycardia; bronchoconstriction; tremors
5-HT _{1B}	HTR1B	CVS, CNS	Cerebral and coronary artery vasoconstriction; increase in BP	increase in aggression
5-HT _{2A}	HTR2A	CVS, CNS	Smooth muscle contraction; platelet aggregation; potential memory impairments; hallucinations; schizophrenia; serotonin syndrome	Insufficient information
5-HT _{2B}	HTR2B	CVS, pulmonary, development	Potential cardiac valvulopathy; pulmonary hypertension	Possible cardiac effects, especially during embryonic development
Vasopressin V _{1A} receptor	AVPR1A	Renal, CVS	Water retention in body; increase in BP; decrease in HR; myocardial	Insufficient information

			fibrosis; cardiac hypertrophy; hyponatraemia	
Acetylcholine receptor subunit $\alpha 1$ or $\alpha 4$	CHRNA1, CHRNA4	CNS, CVS, GI, pulmonary	Paralysis; analgesia; increase in HR; palpitations; nausea; abuse potential	Muscle relaxation; constipation; apnoea; decrease in BP; decrease in HR
Voltage-gated calcium channel subunit α Cav1.2	CACNA1C	CVS	Insufficient information	Vascular relaxation; decrease in BP; decrease in PR interval; possible shortening of QT interval of ECG
Potassium voltage-gated channel, subfamily H member 2 (hERG)	KCNH2	CVS	Insufficient information	Prolongation of QT interval of ECG
Potassium voltage-gated channel KQT-like member 1 and minimal potassium channel MinK	KCNQ1 & KCNE1	CVS	Atrial fibrillation	Long QT syndrome; potential hearing impairment, deafness and GI symptoms
Voltage-gated sodium channel subunit α Nav1.5	SCN5A	CVS	Insufficient information	Slowed cardiac conduction; prolonged QRS interval of ECG
Acetylcholinesterase	ACHE	CVS, GI, pulmonary	Insufficient information	decrease in BP; decrease in HR; increase in GI motility (decrease at high doses); bronchoconstriction; increase in respiratory secretions
Cyclooxygenase 2	PTGS2	Immune, CVS	Insufficient information	Anti-inflammatory activity; anti-mitogenic effects; myocardial infarction; increase in BP; ischaemic stroke; atherothrombosis
Monoamine oxidase A	MAOA	CVS, CNS	Insufficient information	increase in BP when combined with amines such as tyramine; DDI potential; dizziness; sleep disturbances; nausea
Phosphodiesterase 3A	PDE3A	CVS	Insufficient information	increase in cardiac contractility; increase in HR; decrease in BP; thrombocytopaenia; ventricular arrhythmia
Noradrenaline transporter	SLC6A2	CNS, CVS	Insufficient information	increase in HR; increase in BP; increase in locomotor activity; constipation; abuse potential
Serotonin transporter	SLC6A4	CNS, CVS	Insufficient information	increase in GI motility; decrease in upper GI transit; decrease in plasma renin; increase in other serotonin-mediated effects; insomnia; anxiety; nausea; sexual dysfunction

Abbreviations: **HR** heart rate; **BP** blood pressure; **CO** cardiac output.

Table 2: Cardiovascular targets from Table 1 in reference [23]. Those also in Table 1 above are highlighted.

Target	Gene	Possible ADRs
Adenosine A ₁	ADORA1	Bradycardia, atrioventricular block. Renal vasoconstriction.
Adenosine A _{2A}	ADORA2A	Hypotension, coronary vasodilation. Facilitation of platelet aggregation.
Adenosine A ₃	ADORA3	Enhanced mediator release could exacerbate asthma and allergic conditions.
Adrenergic α _{1A}	ADRA1A	Hypertension and positive inotropic effect. Orthostatic hypotension.
Adrenergic α _{1B}	ADRA1B	Orthostatic hypotension.
Adrenergic α _{2A}	ADRA2A	Might inhibit insulin secretion, resulting in hyperglycemia. Hypertension exacerbates heart failure.
Adrenergic α _{2B}	ADRA2B	Hypertension, cardiac ischemia (block), vasoconstriction of arteries. Peripherally exacerbates heart failure, centrally reduces blood pressure.
Adrenergic α _{2C}	ADRA2C	Hypertension, cardiac ischemia. Increased muscular, skeletal blood flow.
Adrenergic β ₁	ADRB1	Positive inotropic and chronotropic effects, ventricular fibrillation. Facilitation of bronchospasm, impairs cardiovascular performance.
Adrenergic β ₂	ADRB2	Facilitates cardiac arrest, bronchodilation. Increased bronchospasm, impairs exercise stress cardiovascular performance.
Angiotensin II AT ₁	AGTR1	Increases blood pressure, cell proliferation and migration, tubular Na ⁺ resorption.
Bradykinin B ₁	BDKRB1	Enhances nociception, inflammation, vasodilation and cough.
Bradykinin B ₂	BDKRB2	Enhances nociception, inflammation, vasodilation and cough.
CGRP	CALCRL	Hypocalcaemia and hypophosphatemia.
Ca channel type L	CACNA1C	Hypotension.
Dopamine D ₁	DRD1	Treatment of Parkinson's disease; induces dyskinesia, extreme arousal, locomotor activation, vasodilation and hypotension. Schizophrenia, neurodegeneration, coordination disorders.
Endothelin ET _a	EDNRA	Might cause vasoconstriction, positive inotropy, cell proliferation (e.g. smooth muscle and mesangial cells) and aldosterone secretion.
Endothelin ET _b	EDNRB	Causes initial vasodepression, vasoconstriction, bronchoconstriction and cell proliferation. Vasodilation, platelet aggregation.
Ghrelin GHSR	GHSR1	Energy homeostasis, GH release, effects on glucose homeostasis, cardiovascular effects.
Histamine H ₃	HRH3	Impairs memory, causes sedation, vasodilation, bronchodilation, negative chronotropy and reduces gastrointestinal motility.
Muscarinic M ₁	CHRM1	Vagal effects, blood pressure changes, secretory functions. Decreases gastric acid secretion.
Muscarinic M ₂	CHRM2	Vagal effects, blood pressure changes. Tachycardia.
Muscarinic M ₃	CHRM3	Vagal effects, blood pressure changes, salivation. Reduces incontinence, bronchoconstriction and gastrointestinal motility. Interferes with ocular accommodation, dry mouth.
Muscarinic M ₄	CHRM4	Vagal effects, blood pressure changes. Facilitation of D1 CNS stimulation.
NE transporter	SLC6A2	Inhibitor increases adrenergic hyperactivity and facilitate α ₁ adrenergic activation.
Nicotinic acetylcholine	CHRNA1	Stimulates autonomic cardiovascular, gastrointestinal functions. Palpitation, orthostatic hypotonia, nausea, sweating, muscle tremor, bronchial secretion. Effects on muscular and vegetative ganglionic functions.
NPY Y ₁	NPY1R	Antidepressant, causes vasoconstriction (venous), inhibits gut motility, gastric emptying, acid secretion, pancreatic exocrine secretions. Anxiogenic, inhibits ischemic brain injury.
K channel (hERG)	KCNH2	QT interval (electrocardiogram) prolongation.
K channel [ATP]	KCNJ11	Hypotension. Hypoglycemia.
5-HT _{2B}	HTR2B	Cardiac valvulopathy.
5-HT ₄	HTR4	Facilitates gastrointestinal transit, mechanical intestinal allodynia. Useful in treatment of irritable bowel syndrome, cardiac arrhythmias.
Na channel (site 2)	SCN5A	Antagonist causes cardiac arrhythmia.
Thromboxane A ₂ TP	TBXA2R	Facilitates vascular, uterine and bronchial constriction, gastrointestinal spasm, allergic inflammation and platelet aggregation. Useful in treatment of chronic productive cough, thrombosis, atherosclerosis.
Vasopressin V _{1A}	AVPR1A	Vasopressor.
Vasopressin V _{1B}	AVPR1B	Vasopressor, anxiogenic.

Also interesting is the set of anti-targets offered for screening by the contract screening company Cerep in their ADR Panel [26]. Here, the targets are annotated with the organ(s) affected, although no interaction mode (*e.g.* agonism vs. antagonism) is given. The set is described as being compiled from ADR databases, literature review and statistical association of targets with ADRs using data generated in-house. Although no other details are provided, the panel has been offered for some time so is presumed to have some level of acceptance within the industry. Targets described as involved in ADRs affecting the cardiovascular system are shown in Table 3; those included in either of the two sets above (*i.e.* in Tables 1 and 2 above) are highlighted.

Table 3: Cardiovascular targets taken from reference [26]. Note that in some cases the names given were unclear and gene names were assigned after inspecting the assay details in the catalogue.

Name	Gene	Name	Gene
5-HT transporter	SLC6A4	COX2	PTGS2
5-HT _{2B}	HTR2B	D ₁	DRD1
5-HT _{2C}	HTR2C	D _{4,4}	DRD4
5-HT _{4e}	HTR4	delta2 (DOP)	OPRD1
5-HT ₇	HTR7	GSK3a	GSK3A
A _{2B}	ADORA2B	H ₂	HRH2
ACE	ACE	hERG	KCNH2
acetylcholinesterase	ACHE	kappa (KOP)	OPRK1
adenyl cyclase	ADCY5	M ₂	CHRM2
alpha _{1A}	ADRA1A	MAO-A	MAOA
alpha _{2B}	ADRA2B	MT ₃ (ML2)	MTNR1A, MTNR1B
AR	AR	Na ⁺ site 2	SCN5A
AT ₁	AGTR1	NE transporter	SLC6A2
ATPase (Na ⁺ /K ⁺)	ATP1A1-4, ATP1B1-4	PDE3A	PDE3A
beta ₁	ADRB1	tyrosine hydroxylase	TH
Ca ²⁺ L (diltiazem site)	CACNA1C	UT	UTS2R

Again, the new targets are often members of families already seen, *e.g.* the serotonin, adenosine and dopamine receptors. Others are novel, however. The literature shows these targets mostly do have roles in the cardiovascular system, although the potential for toxicity is not always obvious:

- Angiotensin Converting Enzyme (ACE): involved in regulating vascular tone; however, ACE inhibitors are well studied and at worst have a slight risk of inducing hypotension [49];
- Adenylate cyclase: cAMP is an important second messenger, so inhibiting production could plausibly have deleterious effects; however, evidence for CVS toxicity seems to be lacking.
- Androgen receptor (AR): androgens are known to mediate cardiomyocyte hypertrophy[50], so toxic effects from agonists in particular are plausible;
- Na⁺/K⁺-ATPase: involved in maintaining cardiomyocyte membrane potential; see the discussion in the 'Ion Channels & Pumps' section below;
- GSK3α: involved in several cardiac signal transduction pathways; see discussion in the 'Kinases' section below;
- Melatonin receptor: melatonin receptor agonists have been shown to have cardiovascular effects [51], although the evidence for toxicity is weak. Note that MT1 & MT2 are the important isoforms in humans, not MT3 as suggested by the Cerep list;
- Tyrosine hydroxylase: can affect norepinephrine levels in cardiac tissue [52], although the evidence for toxic effects of TH inhibition is lacking;
- Urotensin II receptor (UT): Urotensin II is a potent vasoconstrictor [53], so toxic CVS effects from agonists in particular are plausible.

There do thus seem to be plausible mechanisms by which several of the novel anti-targets might induce CVS toxicity, in particular Na^+/K^+ -ATPase blockers, GSK3 α inhibitors and AR and UT agonists (although more confirmatory *in vivo* and/or clinical data would be valuable). The other targets seem to be somewhat more speculative, however, and further research on their utility is required. A pragmatic way of treating these, and any other more speculative targets that may be encountered, would be to include them on a 'long-list', but to prioritize them below those that are judged to have a more solid involvement in toxicity in data gathering or modelling efforts.

1.1.1. Ion Channels & Pumps

As mentioned above, the hERG potassium channel is the most studied of the cardiac ion channels, and the one most commonly associated with arrhythmia [45]. However, other channels are also implicated in cardiac ADRs [54]; for example, the L-type Calcium channel ($\text{Ca}_v1.2$) and the Sodium channel ($\text{Na}_v1.5$) are included alongside hERG ($\text{K}_v11.1$) on all three lists above, showing how important they are believed to be. Indeed, simulation of the effects of blockage of these three channels together has been shown to improve prediction of the risk of Torsades des Points arrhythmias over what is possible considering hERG alone [55].

The ATP-sensitive K^+ channel ($\text{K}_{ir}6.2$) and $\text{K}_v\text{LQT1}$ channel ($\text{K}_v7.1$) are also included on one pharmaceutical company list each, showing these targets are recognized there as having some degree of safety liability. Given the above, it makes sense to include the full complement of cardiac channels, with higher priority being given to those included in the lists discussed above. These are shown in Table 4.

Table 4: Taken from Table 1 in reference [54]. Higher priority targets are highlighted.

Current	Description	AP Phase	Activation Mechanism	Clone	Gene(s)
INa	Sodium current	Phase 0	Voltage, depolarization	Nav1.5	SCN5A
ICa,L	Calcium current, L-type	Phase 2	Voltage, depolarization	Cav1.2	CACNA1C
ICa,T	Calcium current, T-type	Phase 2	Voltage, depolarization	Cav3.1/3.2	CACNA1G,CACNA1H
Ito,f	Transient outward current, fast	Phase 1	Voltage, depolarization	KV 4.2/4.3	KCND2,KCND3
Ito,s	Transient outward current, slow	Phase 1	Voltage, depolarization	KV 1.4/1.7/3.4	KCNA4,KCNA7,KCNC4
IKur	Delayed rectifier, ultrarapid	Phase 1	Voltage, depolarization	KV 1.5/3.1	KCNA5,KCNC1
IKr	Delayed rectifier, fast	Phase 3	Voltage, depolarization	HERG ($\text{K}_v11.1$)	KCNH2
IKs	Delayed rectifier, slow	Phase 3	Voltage, depolarization	$\text{K}_v\text{LQT1}$ ($\text{K}_v7.1$)	KCNQ1
IK1	Inward rectifier	Phase3&4	Voltage, depolarization	Kir 2.1/2.2	KCNJ2,KCNJ12
IKATP	ADP activated K^+ current	Phase1&2	[ADP]/[ATP] increase	Kir 6.2 (SURA)	KCNJ11
IKAch	Muscarinic-gated K^+ current	Phase 4	Acetylcholine	Kir 3.1/3.4	KCNJ3/5
IKP	Background current	All Phases	Metabolism, stretch	TWK-1/2,TASK-1,TRAAK	KCNK1,KCNK6,KCNK3,KCNK4
If	Pacemaker current	Phase 4	Voltage, hyperpolarization	HCN2/4	HCN2,HCN4

Abbreviations: AP = Action Potential.

The Na^+/K^+ -ATPase pump is included on the Cerep list (see Table 3), which implies it is associated with some degree of safety liability. Given this fact, it is also possible that cardiac calcium pumps might also be worth including, as they too have a role in cardiac contractility [56]. However, it is also possible that intracellular targets such as the sarcoplasmic reticulum calcium pumps might not be exposed to compounds to the same extent as channels or pumps in the plasma membrane, depending on the compound's permeability.

That different target or anti-targets might experience different compound exposures depending on differences in location is an important issue, which might need to be borne in mind when interpreting the results of *in vitro* experiments in particular [57]. For example, a compound might bind tightly to a particular target, but have little effect if the free concentration at the target is low.

1.1.2. Kinases

The cardiotoxicity of certain protein kinase inhibitors has emerged as an issue in the field of oncology [58, 59]. A particular problem is that some of the pathways that regulate cancer cell survival are also involved in cardiomyocyte homeostasis and survival. Thus, the toxicity of anti-cancer compounds targeting these pathways is inextricably linked with the desired therapeutic mechanism, *i.e.* it is an 'on-target' effect. In the context of the treatment of a life-threatening cancer, this might be a tolerable risk, and several such compounds are indeed used successfully in the clinic. However, if the disease is less serious, the dosing is chronic and/or there is pre-existing cardiovascular disease then the acceptable risk will be lower. In this case, off-target perturbation of these pathways by insufficiently-selective kinase inhibitors could become a problem [60].

A recent review lists over thirty kinases believed to be of importance in the heart and vasculature, based on the results of various mouse models [61]; these are shown in Table 5. Deciding exactly which of these are important anti-targets is not straightforward. A provisional (and somewhat subjective) short list might be...

- VEGFR and PDGFR β : important in the heart's response to stress.
- PI3K/AKT pathway: regulates cardiomyocyte survival, with AKT particularly important [62].
- CaMK II: regulates calcium homeostasis.
- AMPK: regulates cellular energy metabolism.
- GSK3 α/β : involved in regulating cardiomyocyte growth and stress response.

In light of the importance of mitochondria to this project (see section below), it is interesting to note that many kinase signalling pathways target mitochondria, and inhibition of these pathways may thus have effects on energy metabolism and cell survival [63], in the heart and elsewhere.

It must be remembered that this is an emerging area and more information is needed before a definitive list of kinase cardiovascular anti-targets can be created. In addition, it is possible that inhibition of multiple kinases might be needed to cause a toxic insult to occur, just as in some cases it is required for a therapeutic effect [64]. In this case, the kinase inhibition *profile* of a compound might be more important for toxicity than its activity at any particular kinase [61, 65].

1.1.3. Other target classes

There is a complement of transporters expressed in the heart [66], and there is some evidence that interaction with these transporters can be associated with cardiac toxicities [46]. There is relatively little information about this area, however, and it will not be pursued further at present.

Mitochondria are known to be important in cardiotoxicity [31, 32], which opens up a range of possible anti-targets. This is discussed in a separate section, 'Mitochondria', below.

Table 5: Taken from Table 2 in Reference [61]. Some kinases believed to be particularly important as cardiovascular anti-targets are highlighted.

Kinase(s)	Gene(s)	Role of kinase in heart/vasculature
RAF1/BRAF	BRAF	Anti-apoptotic; preserves LV function under stress. KO: LV dysfunction and HF in the absence of additional stress; DNTG: reduced hypertrophy but LV dysfunction due to cell death
PI3K (p110 α)	PIK3CA	Physiological heart growth; cardiomyocyte survival
PI3K (p110 γ)	PIK3CG	Regulates contractility and pathological hypertrophy
PDK1	PDK1	Cardiomyocyte survival and β -adrenergic responsiveness
AKT1, 2 or 3	AKT1/2/3	Regulators of cardiomyocyte survival, growth and metabolism
mTOR	MTOR	mTORC1 regulates protein synthesis, inhibition leads to energy preservation under stress; mTORC2 regulates AKT activation
AMPK	PRKAA1/2, PRKAB1/2, PRKAG1/2/3	Sensor of energy stress; inhibits mTORC1, preserving energy stores. KO of AMPK α 2 increased hypertrophy and LV dysfunction after TAC
GSK3 α / β	GSK3A/B	Together with AMPK, inhibits mTORC1; deletion of GSK β protective in post-MI remodelling; deletion of GSK3 α leads to HF in setting of stress
CDKs	CDK2/4	CDK2 inhibition reduces ischaemia– reperfusion injury, mediated via effects on retinoblastoma protein
Aurora kinases	AURKA/B/C	M phase regulators
PLKs	PLK1	PLK1 involved in activation of CDC2, chromosome segregation, centrosome maturation, bipolar spindle formation and cytokinesis
PDGFRs	PDGFRA/B	β isoform is crucial in angiogenesis and heart's response to PO
VEGFRs	FLT1/KDR/FLT4	Crucial in angiogenesis and the heart's response to PO; antihypertensive effects
EGFR (ERBB1)	EGFR	Helps to maintain LV function in setting of chronic catecholamine stimulation; mediates pro-survival signalling
ERBB2	ERBB2	Cardiomyocyte survival and homeostasis; maintenance of LV function
KIT	KIT	Promotes CSC and immature cardiomyocyte differentiation; promotes homing to sites of MI, promoting repair.
ABL/ARG	ABL1	Maintains ER homeostasis. LV dysfunction is seen in rodents treated with imatinib
JAK2	JAK2	JAK2 and STAT3 protective in many pathological settings
FAK	PTK2	Antihypertrophic and antifibrotic in heart
DMPK	DMPK	Myotonic dystrophy type 1 is caused by excess repeats of the 3' UTR region of DMPK
LTK	LTK	Activation of LTK results in cardiac hypertrophy and cardiomyocyte degeneration
ROCK	ROCK1/2	Pro-fibrotic and pro-apoptotic in the setting of PO
LKB1	STK11	Activates AMPK which is pro-angiogenic in heart
ERK1/2	MAPK3/1	Generally promotes survival and may modulate physiological (but not pathological) hypertrophy
PKC α	PRKCA	Adverse effects on heart in setting of PO
PKG	PRKG1	One of the four nodal kinases in HF; activated by PDE5 inhibitors; inhibits apoptosis, hypertrophy and β -adrenergic responses
PIM Kinase	PIM1	Pro-survival; activated by AKT; regulated at level of gene expression
CAMKII	CAMK2A	Nodal kinase in HF; pro-hypertrophic; promotes decompensation in setting of PO Mechanism of cardiotoxicity involves regulation of CAMKII gene expression and Ca ²⁺ handling
GRK2, GRK5	ADRBK1/GRK5	Downregulates β -adrenergic signalling through recruitment of β -arrestin
ASK1	MAP3K5	Promotes pathological hypertrophy and remodelling; pro-apoptotic

1.2. Hepatotoxicity

The state of knowledge on hepatotoxicity is rather different to that of cardiotoxicity. While the understanding of basic mechanisms is growing [3, 4], there are fewer unambiguously defined molecular anti-targets in the sense that has been used here. For example, in the consensus panel of 44 core safety targets mentioned above [24], 30 are annotated as having the cardiovascular system as an affected organ (see Table 1), but there are no annotations for the liver. Similarly, in an effort by the FDA to match modes of action (MOA) to adverse effects, the number of cardiac mechanisms [21] identified far outweighed the hepatobiliary mechanisms [20].

This difference presumably reflects the unique function of the liver: its role in the clearance of xenobiotics means it is exposed to high levels of reactive metabolites [67, 68], and these are one of the key drivers of drug-induced liver damage. These reactive species may exert their effects through various mechanisms, such as depletion of glutathione and covalent binding to proteins, lipids and nucleic acids [69]. This covalent binding is generally considered to be non-specific as compared to typical non-covalent interactions, although there are attempts to document and interpret those proteins that are affected [70, 71].

Covalent modification of proteins can trigger apoptosis *via* the intrinsic pathway, or possibly necrosis in severe cases [3]. Covalent modification of proteins can also lead to haptensisation and thus to activation of the immune system [72, 73], possibly leading to liver damage *via* activation of the extrinsic apoptotic pathway. This immunogenic DILI is particularly hard to predict, as it may only manifest in susceptible individuals and is often only apparent post-marketing.

Direct interaction of parent drug or metabolites with mitochondria can also lead to cell death [29]. As mentioned above, mitochondria are important for hepatotoxicity [29, 30] as well as cardiotoxicity, and will be discussed further below.

1.2.1. Transporters

One class of molecular targets in the liver that are particularly important in drug discovery is the transporters [74]. One transporter known to be associated with direct hepatotoxicity is the Bile Salt Export Pump (BSEP), located in the canalicular membrane of hepatocytes. Inhibition of this transporter can result in a build-up of bile acids (BA) in hepatocytes and hence to cholestasis [75, 76]. However, other transporters in the liver are also involved in BA homeostasis and enterohepatic recirculation; four that are believed to be particularly important [77, 78] are shown in Table 6.

Table 6: Some liver transporters important in bile acid homeostasis, from references [77, 78].

Name	Gene	Location	Function
NTCP	SLC10A1	hepatocyte basolateral membrane	extracts BAs from portal blood
BSEP	ABCB11	hepatocyte canalicular membrane	secretes BAs into biliary tract
ASBT	SLC10A2	cholangiocyte apical membrane	extracts BAs from biliary tract
OST α /OST β	SLC51A/B	cholangiocyte basolateral membrane	secretes Bas back into blood

Mutations in MPR2 and MDR3 (as well as BSEP) are implicated in some hereditary cholestatic diseases [79], which would imply they too should be considered as anti-targets.

Beyond this core set, a variety of other transporters are known to have roles in bile handling in the liver [80]; some of these are listed in Table 7, with location and functional annotation. Although the focus here is on the liver, it should also be borne in mind that BA transport occurs in tissues other

than the liver, most notably the ileum and kidney [77]. This might need to be taken into account when, for example, interpreting *in vivo* data or building PK/PD models.

In addition to these transporters, BA homeostasis also relies on the hepatic Na⁺/K⁺-ATPase for maintaining the Na⁺ gradients on which some transporters, such as NTCP, rely; the hepatic CFTR channel is also required, albeit indirectly, for the functioning of some OATP transporters [80].

Table 7: Taken from Table 1 in reference [80]. Some important examples are highlighted, but note that the OSTα/OSTβ heterodimer is not on this list as its role was discovered relatively recently.

Name	Gene	Location(s)	Main function(s)
NTCP	SLC10A1	BH	Main carrier for Na ⁺ -dependent uptake of conjugated bile salt from portal blood.
OATPs	SLC21A	BH	Na ⁺ -independent uptake of unconjugated bile salts and other organic anions. Polyspecific transporters with overlapping substrate affinity that are able to uptake endo- and xeno-biotics.
OCTs	SLC22A	BH	Hepatic uptake of hydrophilic organic cations. Relevant for drug transport.
OATs	SLC22A	BH	Na ⁺ -independent transport of para-aminohippurate, salicylate, acetylsalicylate and methotrexate.
MRP3	ABCC3	BH, BC	Basolateral efflux of biliary constituents including non-sulfated and sulfated bile salts. Preferentially transports glucuronides but not glutathione, S-conjugates or free glutathione. Might play a role in the removal of bile acids from the liver in cholestasis.
MRP4	ABCC4	BH, BC	Mediates glutathione efflux from hepatocytes into blood by co-transport with monoanionic bile salts. Might also function as an overflow pathway during cholestasis. In bile duct cells, might facilitate the return of bile salts from the obstructed bile ducts to the systemic circulation.
MDR1	ABCB1	CH	ATP-dependent excretion of bulky organic cations into bile.
MDR3	ABCB4	CH	Translocation of phosphatidylcholine from inner to outer leaflet of the membrane bilayer. Crucial for biliary phospholipid secretion.
MRP2	ABCC2	CH	Canalicular conjugate export pump previously known as cMOAT. Transports bilirubin diglucuronide, sulfates, glutathione conjugates and various organic anions into bile in an ATP-dependent manner.
BSEP	ABCB11	CH	Mediates ATP-dependent bile salt transport into bile.
ABCG5	ABCG8	CH	'Half ABC transporters' that function as heterodimers to transport sterols into bile. They might also partially mediate biliary cholesterol secretion.
BCRP	ABCG2	CH	'Half ABC transporter' that mediates cellular extrusion of sulfated conjugates.
AE2	SLC4A2	CH, AC	Facilitates bicarbonate secretion into bile and contributes to bile-salt-independent bile flow.
ABST	SLC10A2	AC	Identical to the ileal bile salt transporter. Functions as an uptake mechanism for bile salts, removing them from bile.
FIC1	ATP8B1	CH, AC	Member of the Type IV P-type ATPase family, which functions as an ATP-dependent aminophospholipid translocase. However, FIC1 function is not yet clearly defined. It is mutated in two different disorders: PFIC1 and BRIC.

Abbreviations: BH = Basolateral membrane of hepatocytes; CH = Canalicular membrane of hepatocytes; BC = Basolateral membrane of cholangiocytes; AC = apical membrane of cholangiocytes.

It would seem plausible that inhibition of one or more of these transporters could cause toxic effects due to the disruption of bile homeostasis. However, the extent to which toxicities beyond those associated with BSEP occur is not yet clear.

As well as direct toxic effects, transporters are frequently involved in drug-drug interactions. Their importance in this context is such that a consortium has been formed by various pharmaceutical companies (the ITC) in order to provide guidance on which transporters are of concern and on the methods used to study them [81, 82]. Transporters of interest to the ITC are shown in Table 8. The full list is included, to emphasize that all may be important when considering DDIs. Those expressed in the liver are highlighted, as these are presumably more likely to be important for *e.g.* direct cholestatic hepatotoxicity.

The overlap of transporters considered important for DDIs with those involved in bile acid transport is evident. It is thus conceivable that inhibitors could exert toxic effects both through disruption of BA homeostasis and through DDIs.

Table 8: Compiled from references [81, 82]. Hepatic transporters are highlighted in gray. Transporters described by the ITC as of particular relevance to drug discovery are bolded.

Name	Gene		Name	Gene
OATP1B1	SLCO1B1		MDR1	ABCB1
OATP1B3	SLCO1B3		BCRP	ABCG2
OAT1	SLC22A6		BSEP	ABCB11
OAT3	SLC22A8		MRP2	ABCC2
OCT2	SLC22A2		MRP3	ABCC3
OATP1A2	SLCO1A2		MRP4	ABCC4
OATP2B1	SLCO2B1		MDR3	ABCB4
OCT1	SLC22A1			
PEPT1	SLC15A1			
PEPT2	SLC15A2			
MATE1	SLC47A1			
MATE2-K	SLC47A2			
OST α /OST β	SLC51A/B			

It is interesting in light of the discussion of mitochondria below that there is a complement of transporters largely specific to the mitochondria [83, 84]. The impermeability of the inner mitochondrial membrane means transporters are crucial for the import and export of metabolites, and it is possible interference with these processes could affect mitochondrial function, in the liver and other organs.

Other transporters beyond those mentioned above are known to be expressed in the liver and elsewhere [66]; however, evidence any involvement in toxicity or DDIs is generally lacking and they will not be considered further at present.

Note that a compound may block a transporter or be a substrate for it. It is possible that both modes could lead to toxic effects, albeit by different mechanisms: the first perhaps by altering the disposition of another drug or of an endogenous molecule, the second by altering the disposition of the transported drug. This distinction would need to be kept in mind when gathering, interpreting and modelling drug/transporter data.

1.2.2. Nuclear Receptors

Nuclear receptors (NR) control expression of ADME proteins (XMEs, transporters *etc.*) in response to xenobiotic insult. Interaction of drugs with NRs can therefore have deleterious consequences, most notably DDIs caused by induction of XMEs [85]. This induction can also cause direct liver toxicity, perhaps by increasing the concentration of reactive metabolites and/or reactive oxygen species [4].

However, NRs are also involved with many homeostatic processes in the liver, including bile acid metabolism/bile secretion [86, 87] and lipid metabolism [88]. Because of these regulatory roles, there is interest in these receptors as therapeutic targets for cholestasis and steatohepatitis. However, as with other therapeutic targets, it is conceivable that inappropriate modulation of these receptors (*e.g.* in different disease states) could lead to toxic outcomes and that off-target interaction with NRs is best avoided. Thus, pharmaceutical companies typically screen against some subset of NRs [89], and CROs offer various NR assays [25]. Those NRs with roles in liver metabolism and/or gene induction are listed in Table 10, with the most important highlighted. Note that the

transcription factors AhR and Nrf1 are not NRs, but are often included alongside them because of their closely related roles.

Table 10: Nuclear Receptors with roles in the liver; those believed to be most important for hepatotoxicity are highlighted [25, 86-89].

Name	Systematic Name	Gene
FXR	NR1H4	NR1H4
SHP	NR0B2	NR0B2
PXR	NR1I2	NR1I2
CAR	NR1I3	NR1I3
VDR	NR1I1	VDR
HNF4 α	NR2A1	HNF4A
LRH1	NR5A2	NR5A2
PPAR α	NR1C1	PPARA
PPAR γ	NR1C3	PPARG
LXR α	NR1H3	NR1H3
LXR β	NR1H2	NR1H2
GR	NR3C1	NR3C1
AhR	n/a	AHR
Nrf2	n/a	NRF1

Note that, as with other types of receptors, ligands for NRs can be agonists, antagonists and possibly inverse agonists. Proper annotation of compound-receptor activity data with the mode of interaction is thus crucial for proper interpretation and modeling.

1.3. Other targets

The Cerep ADR Panel [26] contains targets flagged as relevant to liver toxicity as well as heart toxicity, although the lists overlap heavily. The liver anti-targets are shown in Table 11.

Table 11: Liver targets taken from reference [26]. Those that are also cardiovascular anti-targets are highlighted.

Name	Gene	Name	Gene
ACE	ACE	ERK2 (P42mapk)	MAPK1
alpha2A	ADRA2A	ETB	EDNRB
AR	AR	GR	NR3C1
ATPase (Na+/K+)	ATP1A1-4, ATP1B1-4	GSK3a	GSK3A
beta2	ADRB2	H2	HRH2
carbonic anhydrase II	CA2	MAO-A	MAOA
constitutive NOS (endothelial)	NOS3	motilin	MLNR
COX2	PTGS2	Na+ site 2	SCN5A
D4.4	DRD4	NMDA	GRIN1
ERalpha	ESR1		

As with the cardiovascular case, some of these anti-targets or their families have already been identified as being of interest, while others are novel.

Some examples are discussed below:

- The glucocorticoid receptor (GR) is a known anti-target, and the androgen receptor (AR) is also known to play a role in normal liver function and in disease [90]. Estrogen receptor α (ER α) is also expressed in the liver, although any connection with disease and/or DILI seems less well defined than for AR [91].
- The Na⁺/K⁺-ATPase, as noted above, is involved in bile-acid homeostasis. The importance of maintaining correct sodium levels could explain the inclusion of the sodium channel [92].
- Some Angiotensin Converting Enzyme (ACE) inhibitors are known to cause liver injury, though this is rare and not necessarily an on-target effect [93].
- The kinases GSK3 α and ERK2 are plausible hepatic anti-targets, as they are involved in cell growth and survival pathways. Hepatocytes exist in a high-stress environment, so might well be vulnerable to disruption of these pathways. It is notable in this context that these appear on the list of cardiac kinases of concern [61].
- Nitric oxide signaling is important in the liver [94], so it is conceivable that inhibition of eNOS could be detrimental in some circumstances.

Thus, it does appear that the targets examined so far have at least some plausible connection with liver disease, although this by no means confirms their involvement in DILI. With the receptors in the list, it is not even always clear which mode of engagement (*e.g.* agonism vs. antagonism) might be deleterious.

In conclusion, these anti-targets are interesting due to the mechanistic diversity they suggest. However, they will need further literature research to find evidence corroborating their involvement with hepatotoxicity and/or providing mechanistic rationales for their inclusion.

1.4. Xenobiotic Metabolising Enzymes

As noted in the introduction, XMEs are crucial in mediating various toxicities through the production of active metabolites [67, 68, 95] and possibly *via* drug-drug interactions [36]. Thus, understanding their interactions remains vital to any effort at predictive toxicology. These interactions can be diverse: compounds can be substrates (possibly generating reactive metabolites) or inhibitors of one or more enzymes; they can also act as inducers [4] *via* nuclear receptors (see above).

The PharmaADME group, with pharmaceutical industry participation, designed a ‘core list’ of 32 ADME genes designed “to identify predictors of pharmacokinetic variability that could impact drug safety and efficacy in the current drug development process” [35]. This core list includes Phase I and II XMEs and transporters; these are shown in Table 9. Note that this list is not restricted to hepatic species only, for reasons already discussed.

The group also provides an ‘extended list’ of 267 genes, intended to give a complete set of genes associated with drug metabolism [35]. As well as further XMEs and transporter isoforms, the extended list includes ‘modifiers’: these are nuclear receptors responsible for induction, ancillary enzymes such as cytochrome P450 oxidoreductase and other species required for the proper functioning of the ADME machinery.

Although these lists were designed around pharmacogenomics experiments [96], they together serve as a definitive list of ADME-related genes. For example, the core set is largely, and the extended set entirely, a superset of the ADME targets offered by screening companies [97, 98], the ITC transporters of interest [81, 82] and targets identified by regulators as involved in DDIs [36].

Table 9: Taken from reference [36].

Gene Symbol	Full Gene Name	Class
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	Phase I
CYP1A2	cytochrome P450, family 1, subfamily A, polypeptide 2	Phase I
CYP2A6	cytochrome P450, family 2, subfamily A, polypeptide 6	Phase I
CYP2B6	cytochrome P450, family 2, subfamily B, polypeptide 6	Phase I
CYP2C8	cytochrome P450, family 2, subfamily C, polypeptide 8	Phase I
CYP2C9	cytochrome P450, family 2, subfamily C, polypeptide 9	Phase I
CYP2C19	cytochrome P450, family 2, subfamily C, polypeptide 19	Phase I
CYP2D6	cytochrome P450, family 2, subfamily D, polypeptide 6	Phase I
CYP2E1	cytochrome P450, family 2, subfamily E, polypeptide 1	Phase I
CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4	Phase I
CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5	Phase I
DPYD	dihydropyrimidine dehydrogenase	Phase I
GSTM1	glutathione S-transferase M1	Phase II
GSTP1	glutathione S-transferase pi	Phase II
GSTT1	glutathione S-transferase theta 1	Phase II
NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)	Phase II
NAT2	N-acetyltransferase 2 (arylamine N-acetyltransferase)	Phase II
SULT1A1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	Phase II
TPMT	thiopurine S-methyltransferase,	Phase II
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1	Phase II
UGT2B15	UDP glucuronosyltransferase 2 family, polypeptide B15	Phase II
UGT2B17	UDP glucuronosyltransferase 2 family, polypeptide B17	Phase II
UGT2B7	UDP glucuronosyltransferase 2 family, polypeptide B7	Phase II
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	Transporter
ABCC2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	Transporter
ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	Transporter
SLC15A2	solute carrier family 15 (H ⁺ /peptide transporter), member 2	Transporter
SLC22A1	solute carrier family 22 (organic cation transporter), member 1	Transporter
SLC22A2	solute carrier family 22 (organic cation transporter), member 2	Transporter
SLC22A6	solute carrier family 22 (organic anion transporter), member 6	Transporter
SLCO1B1	solute carrier organic anion transporter family, member 1B1	Transporter
SLCO1B3	solute carrier organic anion transporter family, member 1B3	Transporter

An application using this list is the ADME Sarfari, which integrates tissue expression, orthologues (valuable for cross-species extrapolation) and bioassay data for these proteins and provides convenient access *via* a web portal [99].

1.5. Mitochondria

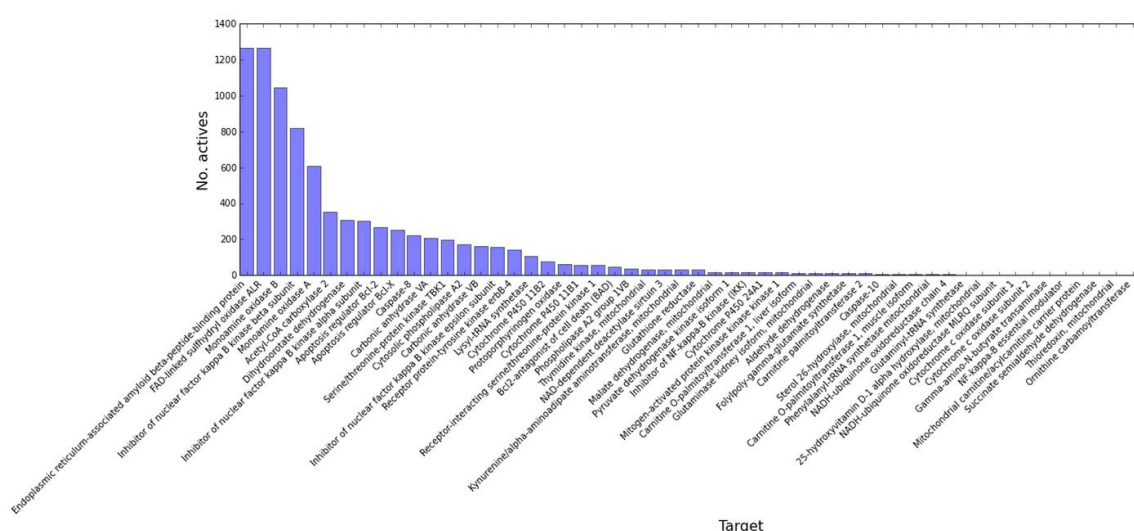
Mitochondria are frequently involved in toxic responses to drugs [29-32]. This is likely to be for two main reasons. First, they play a key role in apoptosis, where they may be effectors of toxic responses triggered by initiating events in which they were not directly involved. Second, they may be targets of toxicants themselves, for example *via* disruption of the citric acid cycle, fatty-acid oxidation or the electron transport chain.

The relevance to HeCaToS is great as mitochondria are particularly important to both the liver [29, 30] and heart [31, 32]; the former because of role in energy homeostasis and the latter because of its energy requirements. In addition, the liver's role in the clearance of xenobiotics means exposure to both parent compounds and active metabolites is likely to be higher than in other tissues.

The involvement in energy homeostasis, lipogenesis *etc.* means mitochondria are of great interest to those studying metabolic diseases. This focus, alongside their roles in apoptosis and toxicity, has led to the creation of several resources integrating various types of 'omics data for mitochondria [100-102]. These resources provide a comprehensive overview of which proteins are found in mitochondria, and which might therefore be the molecular targets of mitochondrial toxins. However, as is often the case with such exhaustive lists of proteins, not all have yet been well characterized and the level of annotation varies widely. Other useful sources of information here are pathway databases such as Reactome [33] and ConsensusPathDB [103], which place individual targets in the biochemical context in which they operate.

The Reactome database was chosen as the basis for an initial investigation of the possibilities for modeling drug-induced mitochondrial dysfunction. This resource contains a particularly rich description of proteins and complexes and the reactions and pathways they are involved in. This can appear complicated when compared with the simple lists of proteins: for example, proteins can appear individually and as part of complexes, complexes might be included in both reduced and oxidized forms and in multiple locations and pathways *etc.* However, this complexity reflects the underlying biology, and provides many extra insights. In addition to enabling mitochondrial modeling, the pathway databases will be useful in further investigation of some of the other anti-targets discussed above.

As an example application, mitochondrial entities from Reactome were mapped to ChEMBL targets, using shared protein chain membership. This enabled the retrieval of ChEMBL data for compounds active against those targets, a prelude to investigating the possibilities of building QSAR models for these targets. The number of compounds active against the various targets is shown in the following histogram...



Note that there are issues that remain to be dealt with here, such as the compatibility of the various assays involved, diversity of the compounds available *etc.* This work is in progress and will be described in detail elsewhere.

DIFFICULTIES

There are several related issues to be considered with regard to the information presented above. The first is that there is likely to be gaps in the coverage of anti-targets. Despite recent advances, aspects of both cardiotoxicity and hepatotoxicity remain poorly understood and this suggests that there are anti-targets and/or mechanisms of toxicity and that remain to be identified.

One way of expanding anti-target coverage might be to use pathway databases to identify other targets on the same pathways as known anti-targets. For example, if antagonism of a cell-surface receptor is known to result in toxicity in some circumstances, then it is plausible that disruption of elements of the signal transduction pathway(s) associated with that receptor might result in a similar phenotype. Similarly, if inhibition of an enzyme on a metabolic pathway results in toxicity, it is possible that inhibition of other enzymes on that pathway might give a similar effect. This sort of thinking is common when attempting to discover new therapeutic targets, and it should also be applicable to anti-target discovery.

There are problems with the approach, however. Importantly, it is likely to introduce false positives: for example, it is well known that there is redundancy built into many signalling pathways [104] and that the effects of inhibiting enzymes on a metabolic pathway might differ depending on whether they catalyse a rate-limiting step or not [105]. In an industrial target discovery setting these hypotheses could be tested by experiment, while in the current context only the literature is available. For example, quantitative models of metabolic networks might help to identify those enzymes that would cause the most disruption if inhibited [106].

Another way of expanding the range of mechanisms covered would be to consult one of the various lists of targets associated with ADRs that have been published [18-21]. In addition, toxicogenomics experiments can identify genes and associated pathways perturbed during a toxic response [107, 108], although the link to anti-targets as discussed here is not always clear.

While these data-driven approaches are attractive, they can only be a starting point in the identification of anti-targets, as confirmatory data (*in vivo* or clinical) or a convincing mechanistic hypothesis would still be required to validate an anti-target. Again, as experiment is not an option here, only the literature is available for further investigating hypotheses.

As noted previously, there are databases of toxicity-associated targets [7, 8] that are useful for annotating known anti-targets but difficult to mine systematically. A related resource which might be useful for this purpose is the Comparative Toxicogenomics Database [109], which links diseases, genes/proteins and compounds. While this began as a resource aimed at environmental toxicants, there is now more of an emphasis on drug-like compounds [110], and the ability to download data means algorithmic mining might be more practical.

Once novel candidate anti-targets are identified, the issue of their validation arises. The amount of information available on different anti-targets differs greatly, and what is actually 'sufficient' in a given context is an open question. Ideally, a comprehensive AOP would be available [9, 75], with unambiguous *in vitro*, *in vivo* or *clinical data* to support each step. However, this will not be available except in rare cases, and decisions (*e.g.* on what to model) will have to be made on incomplete information.

Another issue when considering pharmaceutical anti-targets (or targets) is that of inter-individual variation. In many cases, toxicities only appear after marketing in a small subset of patients. While this can be due to the involvement of the immune system [72, 73], it might also be due to different protein isoforms being present in different individuals [111]. While many such differences will be silent, if they are present in the active site or in a recognition element then they could give rise to differing responses [112-114] to xenobiotics.

It should also be noted that the interactions of xenobiotics with the anti-targets discussed here are at most likely to be risk factors for toxicity. Any actual observable toxic response at a tissue, organ or organism level will most likely vary depending on various factors such as compound exposure, intra-individual genetic variation, pre-existing conditions and co-administered therapeutics.

As a practical issue, the amount of data available for various anti-targets of interest will vary widely. In some cases easily available data for anti-targets of interest may not be sufficient for QSAR model building, for example. The decision will then have to be made as to whether the target is sufficiently important to warrant possibly time-consuming or expensive data-gathering and curation activities.

Finally, as a companion to this report, a table of the anti-targets is being compiled, which will include...

- Useful identifiers to go alongside the gene names used above, such as UniProt and ChEMBL target IDs;
- A prioritisation or ranking, based on the amount of evidence for involvement in toxicity and the potential severity of the response;
- Summary of the data available in ChEMBL and possibly in the wider literature.

This spreadsheet will be made available *via* the project intranet and updated as more data becomes available.

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