**Toxicology-associated targets**

**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 [[1](#_ENREF_1)]. For a variety of reasons, including the multiple mechanisms of hepatotoxicity and many possible confounding factors [[2](#_ENREF_2), [3](#_ENREF_3)], 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 [[4](#_ENREF_4), [5](#_ENREF_5)]. 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 [[6](#_ENREF_6), [7](#_ENREF_7)], 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) [[8](#_ENREF_8)] of an adverse outcome pathway (AOP) [[9](#_ENREF_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](#_ENREF_10)] underpinning a multi-scale approach to toxicity prediction [[11](#_ENREF_11), [12](#_ENREF_12)], an idea and modelling strategy which itself meshes well with the AOP concept [[13](#_ENREF_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](#_ENREF_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 Kd 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](#_ENREF_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](#_ENREF_16), [17](#_ENREF_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](#_ENREF_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](#_ENREF_18)], 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 [[5](#_ENREF_5), [22](#_ENREF_22)], where they are either published by pharmaceutical companies [[23](#_ENREF_23), [24](#_ENREF_24)] or appear in the assay catalogues of contract research organisations (CRO) used by these companies [[25](#_ENREF_25), [26](#_ENREF_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](#_ENREF_27), [28](#_ENREF_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](#_ENREF_29), [30](#_ENREF_30)] and cardiotoxicity [[31](#_ENREF_31), [32](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_35)]. Although they can be involved in direct toxicity [[3](#_ENREF_3)], 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](#_ENREF_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](#_ENREF_37), [38](#_ENREF_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](#_ENREF_39)]. An example would be the use of cell-based assays for measuring drug-induced mitochondrial dysfunction [[40](#_ENREF_40)], or high-content screening assays for DILI [[41](#_ENREF_41)]. Even compendia of hepatotoxic drugs [[42](#_ENREF_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](#_ENREF_43)] and compounds capable of redox-cycling [[44](#_ENREF_44)].

**Cardiovascular Targets**

In some cases, as with hERG and arrhythmias, the link between an anti-target and the associated toxicity is relatively well (although not fully) understood [[45](#_ENREF_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](#_ENREF_46), [47](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_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 protein families already seen (*e.g.* various GPCR subtypes [[49](#_ENREF_49), [50](#_ENREF_50)] or ion channels [[51](#_ENREF_51)]) 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 (*i.e.* homology or mechanism) 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.

The Novartis group also published another version of their cardiac safety panel, which includes some targets not mentioned in the earlier version [[5](#_ENREF_5)]. Most of these are included in the consensus list (Table 1), but Thrombin (F2), the Adenosine transporter (SLC29A1) and the kinases CDK2 and AKT1 (see below) are novel. The list also seperates out the effects of agonists and antagonists, where relevant.

**Table 1.** Cardiovascular targets taken from Table 1 in reference [[24](#_ENREF_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 A2A | 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 |
| α1A-adrenergic receptor | ADRA1A | CVS, GI, CNS | Smooth muscle contraction; increase in BP; cardiac positive ionotropy; 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 |
| α2A-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 |
| β1-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 |
| β2-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 D1 | 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 D2 | 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 H1 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 H2 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 M1 | 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 M2 | 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-HT1B | HTR1B | CVS, CNS | Cerebral and coronary artery vasoconstriction; increase in BP | increase in aggression |
| 5-HT2A | HTR2A | CVS, CNS | Smooth muscle contraction; platelet aggregation; potential memory impairments; hallucinations; schizophrenia; serotonin syndrome | Insufficient information |
| 5-HT2B | HTR2B | CVS, pulmonary, development | Potential cardiac valvulopathy; pulmonary hypertension | Possible cardiac effects, especially during embryonic development |
| Vasopressin V1A receptor | AVPR1A | Renal, CVS | Water retention in body; increase in BP; decrease in HR; myocardial fibrosis; cardiac hypertrophy; hyponatraemia | Insufficient information |
| Acetylcholine receptor subunit α1 or α4 | 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](#_ENREF_23)]. Those also in Table 1 above are highlighted.

|  |  |  |
| --- | --- | --- |
| **Target** | **Gene** | **Possible ADRs** |
| Adenosine A1 | ADORA1 | Bradycardia, atrioventricular block. Renal vasoconstriction. |
| Adenosine A2A | ADORA2A | Hypotension, coronary vasodilation. Facilitation of platelet aggregation. |
| Adenosine A3 | ADORA3 | Enhanced mediator release could exacerbate asthma and allergic conditions. |
| Adrenergic α1A | ADRA1A | Hypertension and positive inotropic effect. Orthostatic hypotension. |
| Adrenergic α1B | ADRA1B | Orthostatic hypotension. |
| Adrenergic α2A | ADRA2A | Might inhibit insulin secretion, resulting in hyperglycemia. Hypertension exacerbates heart failure. |
| Adrenergic α2B | ADRA2B | Hypertension, cardiac ischemia (block), vasoconstriction of arteries. Peripherally exacerbates heart failure, centrally reduces blood pressure. |
| Adrenergic α2C | ADRA2C | Hypertension, cardiac ischemia. Increased muscular, skeletal blood flow. |
| Adrenergic β1 | ADRB1 | Positive inotropic and chronotropic effects, ventricular fibrillation. Facilitation of bronchospasm, impairs cardiovascular performance. |
| Adrenergic β2 | ADRB2 | Facilitates cardiac arrest, bronchodilation. Increased bronchospasm, impairs exercise stress cardiovascular performance. |
| Angiotensin II AT1 | AGTR1 | Increases blood pressure, cell proliferation and migration, tubular Na+ resorption. |
| Bradykinin B1 | BDKRB1 | Enhances nociception, inflammation, vasodilation and cough. |
| Bradykinin B2 | BDKRB2 | Enhances nociception, inflammation, vasodilatation and cough. |
| CGRP | CALCRL | Hypocalcaemia and hypophosphatemia. |
| Ca channel type L | CACNA1C | Hypotension. |
| Dopamine D1 | DRD1 | Treatment of Parkinson's disease; induces dyskinesia, extreme arousal, locomotor activation, vasodilatation and hypotension. Schizophrenia, neurodegeneration, coordination disorders. |
| Endothelin ETa | EDNRA | Might cause vasoconstriction, positive inotropy, cell proliferation (e.g. smooth muscle and mesangial cells) and aldosterone secretion. |
| Endothelin ETb | EDNRB | Causes initial vasodepression, vasoconstriction, bronchoconstriction and cell proliferation. Vasodilatation, platelet aggregation. |
| Ghrelin GHSR | GHSR | Energy homeostasis, GH release, effects on glucose homeostasis, cardiovascular effects. |
| Histamine H3 | HRH3 | Impairs memory, causes sedation, vasodilatation, bronchodilation, negative chronotropy and reduces gastrointestinal motility. |
| Muscarinic M1 | CHRM1 | Vagal effects, blood pressure changes, secretory functions. Decreases gastric acid secretion. |
| Muscarinic M2 | CHRM2 | Vagal effects, blood pressure changes. Tachycardia. |
| Muscarinic M3 | CHRM3 | Vagal effects, blood pressure changes, salivation. Reduces incontinence, bronchoconstriction and gastrointestinal motility. Interferes with ocular accommodation, dry mouth. |
| Muscarinic M4 | CHRM4 | Vagal effects, blood pressure changes. Facilitation of D1 CNS stimulation. |
| NE transporter | SLC6A2 | Inhibitor increases adrenergic hyperactivity and facilitate a1 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 Y1 | NPY1R | Antidepressant, causes vasoconstriction (venous), inhibits gut motility, gastric emptying, acid secretion, pancreatic exocrine secretions. Anxiogenic, inhibits ischemic brain injury. |
| Kchannel (hERG) | KCNH2 | QT interval (electrocardiogram) prolongation. |
| Kchannel [ATP] | KCNJ11 | Hypotension. Hypoglycemia. |
| 5-HT2B | HTR2B | Cardiac valvulopathy. |
| 5-HT4 | 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 A2 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 V1A | AVPR1A | Vasopressor. |
| Vasopressin V1B | 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](#_ENREF_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 [[26](#_ENREF_26), [52](#_ENREF_52)]. 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](#_ENREF_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-HT2B | HTR2B |  | D1 | DRD1 |
| 5-HT2C | HTR2C |  | D4.4 | DRD4 |
| 5-HT4e | HTR4 |  | delta2 (DOP) | OPRD1 |
| 5-HT7 | HTR7 |  | GSK3a | GSK3A |
| A2B | ADORA2B |  | H2 | HRH2 |
| ACE | ACE |  | hERG | KCNH2 |
| acetylcholinesterase | ACHE |  | kappa (KOP) | OPRK1 |
| adenylyl cyclase | ADCY5 |  | M2 | CHRM2 |
| alpha1A | ADRA1A |  | MAO-A | MAOA |
| alpha2B | ADRA2B |  | MT3 (ML2) | MTNR1A, MTNR1B |
| AR | AR |  | Na+ site 2 | SCN5A |
| AT1 | AGTR1 |  | NE transporter | SLC6A2 |
| ATPase (Na+/K+) | ATP1A1-4 & ATP1B1-4 |  | PDE3A | PDE3A |
| beta1 | ADRB1 |  | tyrosine hydroxylase | TH |
| Ca2+ 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.

* Although the 5-HT2B isoform is a cardiovascular antitarget, there is no evidence that 5‑HT2C presents such a risk [[53](#_ENREF_53)].
* The 5-HT7 receptor plays a role in smooth muscle relaxation, and so ligands might be expected to have cardiovascular effects [[54](#_ENREF_54)].
* The Adenosin A2a receptor affects cardiac contractility, so a role in cardiotoxicity is plausible [[55](#_ENREF_55)].
* Angiotensin Converting Enzyme (ACE) is involved in regulating vascular tone; however, ACE inhibitors are well studied in the clinic and at worst have a slight risk of inducing hypotension [[56](#_ENREF_56)].
* Adenylate Cyclase plays an important role in regulating cardiac iontropy and lusitropy, so inhibitors could plausibly have deleterious effects on the heart [[57](#_ENREF_57)].
* Androgens are known to mediate cardiomyocyte hypertrophy, so cardiotoxic effects from androgen receptor (AR) agonists in particular are plausible [[58](#_ENREF_58)].
* Na+/K+‑ATPase is involved in maintaining cardiomyocyte membrane potential; see the discussion in the ‘Ion Channels & Pumps’ section below.
* Clozapine is somewhat selective for the dopamine D4 subtype over other subtypes [[59](#_ENREF_59)], and is associated with cardiotoxicity in the clinic [[60](#_ENREF_60)]. Although the mechanisms of cardiotoxicity aren’t clear, D4 is known to be expressed in the heart [[61](#_ENREF_61)].
* GSK3α is involved in several cardiac signal transduction pathways; see discussion in the ‘Kinases’ section below.
* Melatonin receptor agonists have been shown to have cardiovascular effects, although the evidence for actual toxicity is weak. Note that MT1 & MT2 are the important isoforms in humans, *not* MT3 as suggested by the Cerep list [[62](#_ENREF_62)].
* Tyrosine hydroxylase can affect norepinephrine levels in cardiac tissue, although the evidence for toxic effects of TH inhibition is lacking [[63](#_ENREF_63)].
* Urotensin II known to modulate cardiovascular function is a number of ways, so toxic CVS effects from urotensin II receptor (UT) ligands is plausible [[64](#_ENREF_64)].

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, GSK3α 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.

**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](#_ENREF_45)]. However, various other members of the ion channel superfamily [[65](#_ENREF_65)] are also implicated in cardiac ADRs [[66](#_ENREF_66)]; for example, the L-type Calcium channel (CaV1.2) and the Sodium channel (NaV1.5) are included alongside hERG (KV11.1) 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 [[67](#_ENREF_67)].

The ATP-sensitive K+ channel (Kir6.2) and KVLQT1 channel (KV7.1) 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 ion channels, with higher priority being given to those discussed above. The α-subunits of these channels are shown in Table 4.

**Table 4.** Taken from Table 1 in reference [[66](#_ENREF_66)]. 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 (KV11.1) | KCNH2 |
| IKs | Delayed rectifier, slow | Phase 3 | Voltage, depolarization | KVLQT1 (KV7.1) | 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 [[68](#_ENREF_68)]. 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 [[69](#_ENREF_69)]. For example, a compound might bind tightly to a particular target, but have little effect if the free concentration at the target is low.

**Kinases**

The cardiotoxicity of certain protein kinase inhibitors has emerged as an issue in the field of oncology [[70](#_ENREF_70), [71](#_ENREF_71)]. 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 comopounds 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 [[72](#_ENREF_72)].

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 [[73](#_ENREF_73)]; 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 PDGFRβ: important in the heart’s response to stress.
* PI3K/AKT pathway: regulates cardiomyocyte survival, with AKT particularly important [[74](#_ENREF_74)].
* CaMK II: regulates calcium homeostasis.
* AMPK: regulates cellular energy metabolism.
* GSK3α/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 [[75](#_ENREF_75)], 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 [[76](#_ENREF_76)]. In this case, the kinase inhibition *profile* of a compound might be more important for toxicity than its activity at any particular kinase [[73](#_ENREF_73), [77](#_ENREF_77)].

**Other target classes**

There is a complement of transporters expressed in the heart [[78](#_ENREF_78)], and there is some evidence that interaction with these transporters can be associated with cardiac toxicities[[46](#_ENREF_46)]. There is relatively little information about this area, however, and it will not be pursued further at present.

A comprehensive list of 233 proteins linked to cardiovascular diseases in the literature has been published [[79](#_ENREF_79)]. Many of the antitargets discussed above are included, especially amongst the GPCRs and ion channels. What is particularly interesting, however, is that many enzymes of various classes are also included. While not all of these will be relevant for cardiotoxicity, this list could provide an excellent starting point for investigating further cardiovascular antitargets.

Finally, mitochondria are known to be important in cardiotoxicity [[31](#_ENREF_31), [32](#_ENREF_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 [[73](#_ENREF_73)]. 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 (p110α) | PIK3CA | Physiological heart growth; cardiomyocyte survival |
| PI3K (p110γ) | 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 AMPKα2 increased hypertrophy and LV dysfunction after TAC |
| GSK3α/β | GSK3A/B | Together with AMPK, inhibits mTORC1; deletion of GSKβ protective in post-MI remodelling; deletion of GSK3α 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 |
| PKCα | 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 Ca2+ handling |
| GRK2, GRK5 | ADRBK1, GRK5 | Downregulates β‑adrenergic signalling through recruitment of β‑arrestin |
| ASK1 | MAP3K5 | Promotes pathological hypertrophy and remodelling; pro-apoptotic |

**Hepatotoxicity**

The state of knowledge on hepatotoxicity is rather different to that of cardiotoxicity. While the understanding of basic mechanisms is growing [[2](#_ENREF_2), [3](#_ENREF_3)], there seem to be 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](#_ENREF_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](#_ENREF_21)] identified far outweighed the hepatobiliary mechanisms [[20](#_ENREF_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[[80](#_ENREF_80), [81](#_ENREF_81)], 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 [[82](#_ENREF_82)]. 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 [[83](#_ENREF_83), [84](#_ENREF_84)].

Covalent modification of proteins can trigger apoptosis *via* the intrinsic pathway, or possibly necrosis in severe cases [[2](#_ENREF_2)]. Covalent modification of proteins can also lead to haptenisation and thus to activation of the immune system [[85](#_ENREF_85), [86](#_ENREF_86)], 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](#_ENREF_29)]. As mentioned above, mitochondria are important for hepatotoxicity [[29](#_ENREF_29), [30](#_ENREF_30)] as well as cardiotoxicity, and will be discussed further below.

**Transporters**

One class of molecular targets in the liver that are particularly important in drug discovery is the transporters [[87](#_ENREF_87)]. 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 [[88](#_ENREF_88), [89](#_ENREF_89)]. However, other transporters in the liver are also involved in BA homeostasis and enterohepatic recirculation; four that are believed to be particularly important [[90](#_ENREF_90), [91](#_ENREF_91)] are shown in Table 6.

**Table 6**. Some liver transporters important in bile acid homeostasis, from references [[90](#_ENREF_90), [91](#_ENREF_91)].

|  |  |  |  |
| --- | --- | --- | --- |
| **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 | SLCI0A2 | cholangiocyte apical membrane | extracts BAs from biliary tract |
| OSTα/OSTβ | 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 [[92](#_ENREF_92)], 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 [[93](#_ENREF_93)]; 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 also occurs in tissues other than the liver, most notably the ileum and kidney [[90](#_ENREF_90)]. 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[[93](#_ENREF_93)].

**Table 7**. Taken from Table 1 in reference [[93](#_ENREF_93)]. 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 | SLCO1B1/1B3/2B1 | 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. |
| OCT | SLC22A1 | BH | Hepatic uptake of hydrophilic organic cations. Relevant for drug transport. |
| OATs | SLC22A7/9 | 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. |
| ASBT | 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 [[94](#_ENREF_94), [95](#_ENREF_95)]. 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 hepatotoxicity (by whatever mechanism).

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**. Hepatic transporters from the ITC reviews [[94](#_ENREF_94), [95](#_ENREF_95)]. Those considered to be of particular relevance to drug discovery are bolded.

|  |  |
| --- | --- |
| **Name** | **Gene** |
| **OATP1B1** | **SLCO1B1** |
| **OATP1B3** | **SLCO1B3** |
| OATP1A2 | SLCO1A2 |
| **OATP2B1** | **SLCO2B1** |
| OCT1 | SLC22A1 |
| **MATE1** | **SLC47A1** |
| MATE2-K | SLC47A2 |
| **MDR1** | **ABCB1** |
| **BCRP** | **ABCG2** |
| **BSEP** | **ABCB11** |
| **MRP2** | **ABCC2** |
| MRP3 | ABCC3 |
| MRP4 | ABCC4 |
| MDR3 | ABCB4 |
| OAT2 | SLC22A7 |
| OAT7 | SLC22A9 |
| NTCP | SLC10A1 |
| OSTα/β | SLC51A/B |
| MRP6 | ABCC6 |
| **ENT1/2** | **SLC29A1/2** |

It is interesting in light of the discussion of mitochondria below that there is a complement of transporters largely specific to the mitochondria [[96](#_ENREF_96), [97](#_ENREF_97)]. 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[[78](#_ENREF_78)]; 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.

**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[[98](#_ENREF_98)]. This induction can also cause direct liver toxicity, perhaps by increasing the concentration of reactive metabolites and/or reactive oxygen species[[3](#_ENREF_3)].

However, NRs are also involved with many homeostatic processes in the liver, including bile acid metabolism/bile secretion [[99](#_ENREF_99), [100](#_ENREF_100)] and lipid metabolism [[101](#_ENREF_101)]. 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[[102](#_ENREF_102)], and CROs offer various NR assays [[25](#_ENREF_25), [26](#_ENREF_26)]. Nuclear receptors with roles in the liver are listed in Table 9, with those judged most likely to be involved in hepatoxicity highlighted [[99-101](#_ENREF_99)]. Note that the transcription factors AhR and Nrf1 are not NRs, but are often discussed alongside them because of their closely related roles.

**Table 9**. Nuclear Receptors with roles in the liver [[99](#_ENREF_99)]; those believed to be most important for hepatotoxicity are highlighted [[100](#_ENREF_100), [101](#_ENREF_101)].

|  |  |  |
| --- | --- | --- |
| **Name** | **Systematic Name** | **Gene** |
| FXR | NR1H4 | NR1H4 |
| SHP | NR0B2 | NR0B2 |
| PXR | NR1I2 | NR1I2 |
| CAR | NR1I3 | NR1I3 |
| VDR | NR1I1 | VDR |
| HNF4α | NR2A1 | HNF4A |
| LRH1 | NR5A2 | NR5A2 |
| PPARα | NR1C1 | PPARA |
| PPARγ | NR1C3 | PPARG |
| LXRα | NR1H3 | NR1H3 |
| LXRβ | NR1H2 | NR1H2 |
| GR | NR3C1 | NR3C1 |
| RARα | NR1B1 | RARA |
| AhR | n/a | AHR |
| Nrf2 | n/a | NFE2L2 |

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.

**Other targets**

The Cerep ADR Panel[[26](#_ENREF_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 10.

**Table 10.** Liver targets taken from reference [[26](#_ENREF_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:

* Liver injury from ACE inhibitors has been reported but it is rare and there does not seem to be evidence that it is an on-target effect [[103](#_ENREF_103)].
* Although there is some evidence that α2A receptor agonists might potentiate hepatotoxicity of co-administered xenobiotics [[104](#_ENREF_104)], there seems to be little or no evidence on-target hepatotoxicity associated with this receptor.
* Androgen signalling *via* the androgen receptor (AR) has been shown to suppress the development of steatosis [[105](#_ENREF_105)]; it is thus plausible that inhibition of this process by xenobiotics might be deleterious, at least in some disease states.
* The Na+/K+-ATPase, as noted above, is involved in bile-acid homeostasis [[93](#_ENREF_93)]. It is thus plausible that interference with the activity of this pump could lead to liver damage.
* Although there is some evidence that β2 receptor agonists might potentiate hepatotoxicity of co-administered xenobiotics [[104](#_ENREF_104)], there seems to be little evidence of direct DILI associated with either agonists [[106](#_ENREF_106)] or antagonists [[107](#_ENREF_107)]. As these are very widely used classes of drugs, any on-target hepatotoxicity should be readily apparent; that it is not suggests that this target is unlikely to be a genuine DILI liability.
* Some carbonic anhydrase (CA) inhibitors, such as acetazolamide, have been associated with idiosyncratic DILI, albeit rarely [[108](#_ENREF_108)]. However, this appears to be a class effect associated with the thiadiazolone moiety as opposed to on-target toxicity. In addition, CA inhibitors are contraindicated for patients with severe liver disease as the diuretic effect can lead to hypokalaemia and hence induce coma [[109](#_ENREF_109)]. While this could be important clinically, it does not mean CA inhibitors are likely to be hepatotoxic in non-vulnerable patient populations.
* Nitric oxide signaling is important in the liver [[110](#_ENREF_110)], so it is conceivable that inhibition of eNOS could be detrimental in some circumstances.
* NSAIDs are very widely used drugs and, while cases of idiosyncratic hepatotoxicity have been reported [[111](#_ENREF_111)], there does not seem to be much evidence of on-target hepatotoxicity associated with COX2.
* There does not appear to be clinical evidence of on-target hepatotoxicity for dopamine receptor ligands in general. Compounds selective for the D4 isoform are uncommon, so data here is particularly scare. However, apomorphine is a faily potent D4 agonist (also active at other isoforms) that is, at most, rarely associated with DILI [[112](#_ENREF_112)].
* Estrogen receptor α (ERα) is expressed in the liver, although, by contrast with the AR, there does not seem to be any obvious connection with liver disease [[113](#_ENREF_113)].
* The kinases ERK2 and GSK3α and 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 [[73](#_ENREF_73)].
* There is some evidence of a risk of hepatoxicity in patients receiving Endothelin receptor antagonists [[114](#_ENREF_114)]. However, there seems to be little or no evidence that this is due to on-target activity at ETB: a more plausible suggestion is that the sulphonamide moiety in Bosetan, the most common ET inhibitor in use, might be responsible for the observed DILI.
* The glucocorticoid receptor (GR) is a known anti-target, as noted above.
* Histamine H2 receptor antagonists are extremely widely used and well tolerated [[115](#_ENREF_115)]: there seems to be no reason to suspect on-target hepatotoxicity in this case. Relatively little information on agonists is available.
* Monoamine Oxidase (MAO) inhibitors have historically been associated with DILI; however, this is likely to have been due to reactive metabolites related to the presence of a hydrazine moiety in many earlier examples, and not to on-target hepatotoxicity [[116](#_ENREF_116)]
* There seems top be little evidence linking motilin receptor ligands to hepatotoxicity. The macrolide antibiotic erythromycin is associated with a low rate DILI , which is believed to occur *via* an allergic mechanism; however, the frequency of use means such cases are not uncommon [[117](#_ENREF_117)]. A hemiketal metabolite of drug is a motilin receptor agonist, responsible for the well-known GI side-effects [[118](#_ENREF_118)]. However, there is no evidence linking this activity to the hepatotoxicity.
* The epithetial sodium channel ENaC works with the Na+/K+-ATPase pump in maintaining sodium ion homeostasis [[119](#_ENREF_119)], which as has been noted above, is important in bile acid homeostasis. However, this assay is based on the voltage-gated sodium channel. While the multi-target antiarrythmic drug Dronedarone has some activity at this channel, and has a mild association with clinical DILI [[120](#_ENREF_120)], there is no reason to believe this is due to the sodium channel activity.
* There seems to be no evidence that activity at the NMDA receptor is responsible for DILI. The NMDA receptor antagonist Memantine is only rarely associated with liver damage in clinical use, and there is no reason to believe this is an on-target effect [[121](#_ENREF_121)].

Thus, it appears that some of these targets do appear to be legitimate hepatotoxicity antitargets, and for others there is at least a plausible mechanism by which they might be involved in DILI. Some caution should be exercised in the latter cases, however. For example, the involvement of CA inhibitors in hepatotoxicity is genuine but indirect and these compounds are unlikely to induce DILI in those without serious preexisting liver disease. It might thus be somewhat misleading to characterize such a target as a hepatotoxicity anti-target.

Furthermore, for several targets here the connection with hepatotoxicity is, at best, very weak; this seems to be particularly the case with the GPCRs in the list. Several of these are targets of very widely used drugs, and any on-target hepatotoxicity should be very evident. However, a sufficiently large patient population means there may be many cases of idiosyncratic toxicity observed, even though the actual rate is very low. In addition, idiosyncratic toxicities are generally not on-target effects of a parent drug, but mediated by metabolites through a variety of mechanisms [[122](#_ENREF_122)]. Thus, while there may be many cases of DILI recorded against a drug with a particular therapeutic target, that target may not in reality be associated with hepatotoxicity in any meaningful way.

In conclusion, this list of hepatotoxicity anti-targets is interesting due to the mechanistic diversity it suggests. However, in many cases, further literature research would be required to find evidence corroborating their involvement with hepatotoxicity and/or providing mechanistic rationales for their inclusion.

**Xenobiotic Metabolising Enzymes**

As noted in the introduction, XMEs are crucial in mediating various toxicities through the production of active metabolites [[80](#_ENREF_80), [81](#_ENREF_81), [123](#_ENREF_123)] and possibly *via* drug-drug interactions[[36](#_ENREF_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 [[3](#_ENREF_3)] *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](#_ENREF_35)]. This core list includes Phase I and II XMEs and transporters; these are shown in Table 11. 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](#_ENREF_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 [[124](#_ENREF_124)], 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[[125](#_ENREF_125), [126](#_ENREF_126)], the ITC transporters of interest [[94](#_ENREF_94), [95](#_ENREF_95)] and targets identified by regulators as involved in DDIs [[36](#_ENREF_36)].

**Table 11**. Taken from reference [[36](#_ENREF_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 [[127](#_ENREF_127)].

**Mitochondria**

Mitochondria are frequently involved in toxic responses to drugs [[29-32](#_ENREF_29)]. This is likely to be for two main reasons. First, they play a key role in apopotosis, 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](#_ENREF_29), [30](#_ENREF_30)] and heart [[31](#_ENREF_31), [32](#_ENREF_32)]; the former because of role in energy homeostatis 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 [[128-130](#_ENREF_128)]. 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](#_ENREF_33)] and ConsensusPathDB [[131](#_ENREF_131)], 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.

**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 likely to introduce false positives: for example, it is well known that there is redundancy built into many signalling pathways [[132](#_ENREF_132)] and that the effects of inhibiting enzymes on a metabolic pathway might differ depending on whether they catalyse a rate-limiting stepor not [[133](#_ENREF_133)]. 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 [[134](#_ENREF_134)].

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](#_ENREF_18)]. In addition, toxicogenomics experiments can identify genes and associated pathways peturbed during a toxic response [[135](#_ENREF_135), [136](#_ENREF_136)], 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 [[6](#_ENREF_6), [7](#_ENREF_7)] 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 [[137](#_ENREF_137)], 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 [[138](#_ENREF_138)], 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](#_ENREF_9), [88](#_ENREF_88)], 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[[85](#_ENREF_85), [86](#_ENREF_86)], it might also be due to different protein isoforms being present in different individuals [[139](#_ENREF_139)]. 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 [[140-142](#_ENREF_140)] 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.

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