pharmaceutics

MDPI

Review

Role of Hepatocyte Transporters in Drug-Induced Liver Injury (DILI)—In Vitro Testing

Péter Tátrai [1], Franciska Erdő [2] and Péter Krajcsi [1,3,4,*]

[1] SOLVO Biotechnology, Charles River Laboratories Hungary, H-1117 Budapest, Hungary
[2] Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, H-1088 Budapest, Hungary
[3] Department of Morphology and Physiology, Faculty of Health Sciences, Semmelweis University, H-1081 Budapest, Hungary
[4] Habilitas Kft, H-1025 Budapest, Hungary
* Correspondence: peter.krajcsi@habilitas.eu

Abstract: Bile acids and bile salts (BA/BS) are substrates of both influx and efflux transporters on hepatocytes. Canalicular efflux transporters, such as BSEP and MRP2, are crucial for the removal of BA/BS to the bile. Basolateral influx transporters, such as NTCP, OATP1B1/1B3, and OSTα/β, cooperate with canalicular transporters in the transcellular vectorial flux of BA/BS from the sinusoids to the bile. The blockage of canalicular transporters not only impairs the bile flow but also causes the intracellular accumulation of BA/BS in hepatocytes that contributes to, or even triggers, liver injury. In the case of BA/BS overload, the efflux of these toxic substances back to the blood via MRP3, MRP4, and OST α/β is considered a relief function. FXR, a key regulator of defense against BA/BS toxicity suppresses de novo bile acid synthesis and bile acid uptake, and promotes bile acid removal via increased efflux. In drug development, the early testing of the inhibition of these transporters, BSEP in particular, is important to flag compounds that could potentially inflict drug-induced liver injury (DILI). In vitro test systems for efflux transporters employ membrane vesicles, whereas those for influx transporters employ whole cells. Additional in vitro pharmaceutical testing panels usually include cellular toxicity tests using hepatocytes, as well as assessments of the mitochondrial toxicity and accumulation of reactive oxygen species (ROS). Primary hepatocytes are the cells of choice for toxicity testing, with HepaRG cells emerging as an alternative. Inhibition of the FXR function is also included in some testing panels. The molecular weight and hydrophobicity of the drug, as well as the steady-state total plasma levels, may positively correlate with the DILI potential. Depending on the phase of drug development, the physicochemical properties, dosing, and cut-off values of BSEP IC_{50} $\leq$ 25–50 μM or total $C_{ss,plasma}$/BSEP $IC_{50} \geq 0.1$ may be an indication for further testing to minimize the risk of DILI liability.

Keywords: hepatotoxicity; DILI; cholestasis; BSEP; transporters; in vitro ADME

check for updates

Citation: Tátrai, P.; Erdő, F.; Krajcsi, P. Role of Hepatocyte Transporters in Drug-Induced Liver Injury (DILI)—In Vitro Testing. *Pharmaceutics* **2023**, *15*, 29. https://doi.org/10.3390/pharmaceutics15010029

Academic Editor: Ian Derek Kerr

Received: 5 December 2022
Revised: 16 December 2022
Accepted: 20 December 2022
Published: 22 December 2022

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Membrane transporters comprise two large superfamilies, the solute carrier (SLC) superfamily and the ATP-binding cassette (ABC) transporter superfamily. The SLC superfamily typically facilitates the influx of substrates, but as most SLCs are exchangers, they may also operate as efflux transporters [1]. Mechanistically, ABC transporters are simpler, with most of them being primary active transporters that fulfil an efflux function.

Some transporters are better known for their roles in mediating the absorption–distribution–metabolism–excretion (ADME) of xenobiotics, including drugs [2]. However, even these transporters have important physiological substrates whose homeostasis may be impaired in the case of transporter inhibition. Such physiological substrates, if their plasma levels change in parallel with those of the substrate drugs, may serve as biomarkers

and surrogate probes in drug–drug interaction studies. This aspect has been extensively reviewed elsewhere [3–5] and will not be covered in this review.

Some hepatocytic transporters, on the other hand, have mostly been investigated for their roles in the homeostasis of a special class of physiological substrates: bile acids and bile salts. The impaired homeostasis of bile components—bilirubin being another classical example [6]—may lead to toxicity or other adverse events, and elevated plasma levels of such molecules may indicate the toxic potential of drugs. The homeostasis of physiological substrates is a complex process involving the extensive interplay of transporters and enzymes. Elimination through the liver is mediated by basolateral influx transporters and apical efflux transporters, whereas reabsorption occurs via enterohepatic or cholehepatic shunts. A third transport pathway provided by basolateral efflux functions is a relief pathway that protects hepatocytes from the intracellular accumulation of physiological substrates to tissue-toxic levels and ultimately redirects these substrates toward urinary excretion.

2. Cholestasis—A Major Form of Drug-Induced Liver Injury

Drug-induced liver injury (DILI) is the leading cause of drug development termination and drug withdrawal [7,8]. The package inserts of many marketed drugs display black box warnings about the risk of hepatic injury. Drugs may cause liver injury shortly after administration in a dose-dependent manner that is labelled as "intrinsic" hepatotoxicity. Idiosyncratic hepatotoxicity is more challenging as it may occur weeks or months after treatment [9]. Idiosyncratic DILI was traditionally considered dose-independent, but this concept has been challenged lately [10]. Mechanistically, a drug may cause cholestatic, hepatocellular, or mixed liver injury [11,12]. Cholestatic and mixed cases constitute 20–40% and 12–20% of DILI cases, respectively [13]. Based on these and additional clinical data, different scoring systems of DILI have been proposed [14].

Cholestasis is defined as decreased bile flow. Depending on the cause and site of the disturbance, cholestasis may be classified as intrahepatic if the dysfunction (e.g., inflammation) is intrinsic to the liver or extrahepatic if the extrahepatic portion of the biliary tree is obstructed. At the level of clinical laboratory findings, according to the Council for International Organizations of Medical Sciences (CIOMS) criteria, liver injury is designated as cholestatic if (i) serum alkaline phosphatase (AP) activity is elevated to >2-fold of the upper limit of normal, or (ii) the alanine amino transferase (ALT) serum activity to AP serum activity ratio (ALT/AP) is $\leq$2 [11]. The increased release of AP into the serum was shown to be induced by elevated intracellular bile salt levels characteristic of cholestasis [15]. Cholestasis has long been associated with defects of the transporter function as inherited forms of cholestasis, referred to as various types of progressive familial intrahepatic cholestasis (PFIC), are linked with mutations in transporter-coding genes. The PFIC type 1 is linked with mutations in *ATP8B1*, the PFIC type 2 with mutations in *ABCB11*/BSEP, and the PFIC type 3 with mutations in *ABCB4*/MDR3. Interestingly, children with different types of PFIC display a different spectrum of symptoms: failure to thrive is common in PFIC1, elevated ALT is common in PFIC2 (BSEP deficiency), and thrombocytopenia is common in PFIC3 (MDR3 deficiency) [16].

2.1. Bile Salt Transporters

Bile acids (BA) synthesized de novo in the liver undergo amidation to form bile salts (BS), also called monovalent bile acids. Most bile acids are then conjugated with glycine in healthy humans and taurine in rodents. Both BA and BS can undergo Phase II metabolism to yield sulfated or glucuronidated BA/BS. The Phase II conjugates of monovalent BS are also called divalent bile salts. The transport of BA/BS into hepatocytes is mediated by the sinusoidal influx transporters sodium taurocholate co-transporting polypeptide (NTCP)/solute carrier 10A1 (*SLC10A1*), organic anion transporting polypeptide 1B1 (OATP1B1)/solute carrier organic anion transporter 1B1 (*SLCO1B1*), and OATP1B3/*SLCO1B3*. Canalicular transport is mainly mediated using a bile salt export pump (BSEP)/*ABCB11* and mul-

tidrug resistance-associated protein 2 (MRP2)/*ABCC2*. MRP3/*ABCC3* and MRP4/*ABCC4* provide the sinusoidal efflux function. The heterodimeric organic solute transporter (OSTα/β)/*SLC51A/B* is a facilitative transporter and may mediate transport into as well as out of the hepatocyte [17].

2.1.1. NTCP

NTCP is one of the several membrane transporters highly expressed on the sinusoidal (basolateral) membrane of hepatocytes [18]. NTCP is the primary influx transporter for the sodium-dependent BS uptake from portal blood [19,20]. The cloning of human NTCP [21] greatly facilitated the identification of its substrates and inhibitors [22–27].

The significance of NTCP was clearly demonstrated in a study on knockout mice that displayed hypercholanemia mainly composed of conjugated BS. Several variants described in humans [28,29] showed the impaired transport of bile salts in vitro. The leading clinical phenotype was the elevation of the plasma BS levels that could be mild even in homozygotes [30] and compound heterozygotes [31]. Heterozygotes for the c.800C > T (p.S267F) allele showed no alteration in ursodeoxycholic acid (UDCA) and glycoursodeoxycholic acid (GUDCA) pharmacokinetics after UDCA dosing [32]. This variant displayed about an eight-fold decreased clearance in vitro, resulting from an eight-fold increase in K_m and an unchanged V_{max} for taurocholate [29].

A case report described three children with hypercholanemia persisting beyond 1 year who were shown to be homozygotes for this mutation [33]. The non-synonymous c.755G > A polymorphism (p.R252H) resulted in extremely elevated plasma BS levels, but no cholestasis, jaundice, or liver dysfunction were observed [34,35]. This mild phenotype may be due to a shift in the BS profile towards more soluble and less hepatotoxic sulfated BS which was observed in both *SLC10A1* knockout mice and p.S267F humans [36].

In vitro drug inhibition studies have almost exclusively used taurocholate (TC) as the probe substrate, and the NTCP variants show striking differences in the transport of taurocholate and rosuvastatin [28,29]. In healthy humans, the plasma levels of TC are much lower relative to glycochenodeoxycholate (GCDC) [37], whereas in cholestasis, the TC levels may even exceed those of GCDC in the plasma. Nevertheless, the more hydrophobic GCDC is transported by NTCP with a significantly greater intrinsic clearance compared to TC [38]. Under specific circumstances, the drug-mediated inhibition of NTCP may protect against cholestasis; for example, bosentan, a dual inhibitor of BSEP and NTCP, was cholestatic in humans but non-cholestatic in rats due to the significantly more potent inhibition of rat Ntcp compared to human NTCP [39].

Of note, NTCP interacts with the scaffolding protein four-and-a-half LIM-domain 2 (FHL2) [40]. The expression of FHL2 suppresses NTCP activity, and FHL2 deficiency aggravates cholestasis in mice.

2.1.2. OATP1B1/OATP1B3

OATP1B1 and OATP1B3 are influx transporters localized in the sinusoidal membrane of hepatocytes. Unlike NTCP, OATP1B1 and OATP1B3 are broad substrate specificity transporters with many drug substrates [41], which implies that drugs inhibiting these transporters are quite common, too.

OATP1B1 is the main transporter mediating sodium-independent BS uptake [42,43], but OATP1B3 and its splice variant OATP1B3-1B7 [44] have also been shown to transport BA/BS [45]. The BS transport profiles of OATP1B1 and OATP1B3 have only recently been characterized [43,45–47]. All studies have confirmed the efficient transport of conjugated BA; additionally, OATP1B1 displayed particularly high intrinsic clearance for sulfated BA/BS derivatives. These findings support the use of sulfated and/or glucuronidated BS as biomarkers of the genetic or chemical inhibition of OATP1B1 and OATP1B3 [45,48,49] and, given the increased production of sulfated BA/BS in cholestasis [50], underline the importance of OATP1B1 and OATP1B3 in BA/BS clearance under cholestatic conditions.

Although reports about the effect of *SLCO1B1* polymorphisms on fasting total BA plasma levels have been contradictory [51,52], elevated unconjugated BA levels were observed in *Slco1a/1b* knockout mice [53]. Follow-up studies utilizing knockout models and Ntcp inhibition using Myrcludex showed a more significant role of Ntcp in the hepatic uptake of conjugated BS [54].

2.1.3. BSEP

BSEP plays a pivotal role in the canalicular transport of BA/BS [25]. In humans, the impairment of the gene encoding BSEP, *ABCB11*, causes PFIC-II [55]. Interestingly, BSEP knockout mice display a mild hypercholanemia only, instead of the severe phenotype observed in humans [56]. This is due to compensatory changes such as the increased production of tetrahydroxy BA and upregulation of alternative transport pathways mediated by P-gp (Mdr1a/b) and Mrp4. Bsep/Mdr1a/Mdr1b triple knockouts showed a more severe phenotype [57].

The chemical inhibition of BSEP leads to decreased canalicular efflux and accumulation of BS in the hepatocyte [58]. Unlike non-hepatotoxic BS, hepatotoxic BS damage the inner mitochondrial membrane and precipitate mitochondrial permeability transition [59]. This effect, however, may be experimental system-dependent as isolated mitochondria and HepG2 cells but not HepaRG cells show the direct mitochondrial toxicity of bile salts [60].

2.1.4. MRP2/ABCC2

In hepatocytes, MRP2 is expressed in the canalicular membrane. MRP2/Mrp2 has been linked to cholestasis as a reduction in bile flow was seen in rats upon loss-of-function mutations or the chemical inhibition of Mrp2 [61,62]. However, serum bile acid concentrations do not increase in patients with Dubin–Johnson syndrome, a hereditary MRP2 deficiency [63,64]. A reduction in BS-independent bile flow due to decreased MRP2/Mrp2 function may partly be attributed to the impaired secretion of glutathione (GSH), an MRP2/Mrp2 substrate that is also co-transported with other substrates [65–67]. MRP2 has been shown to transport some monovalent bile salts such as glycocholate (GC) and tauroursodeoxycholate but not taurocholate [68,69]. Rat Mrp2 has been shown to transport TC, albeit at a low rate, as well as GC and 3-sulfo-taurolithocholate (3S-TLC) [70].

2.1.5. MRP3/ABCC3

MRP3 is a major efflux transporter expressed on the sinusoidal membrane of hepatocytes [71]. It is generally considered a relief transporter that effluxes endobiotics and xenobiotics into blood [66]. In rats, Mrp3 expression in the liver is increased upon bile duct ligation [72], and Mrp3 expression has been shown to correlate with the sinusoidal efflux of taurocholate [73]. The role of Mrp3 in BA/BS transport in mice is controversial as plasma BA levels did not decrease in a statistically significant manner in *Abcc3* knockout mice [74,75]. Liver BA levels, however, increased by about 50% in *Abcc3* knockouts [75].

In patients with obstructive cholestasis due to gallstones, MRP3 expression was increased 3.4-fold and 4.6-fold at the mRNA and protein levels, respectively [76], which implies an important role for MRP3 in protection against BA/BS overload. Both rat Mrp3 and human MRP3 have been shown to transport BA: whereas GC and 3S-TLC were identified as common substrates, TC was transported by rat Mrp3 only [77,78]. A detailed profiling and kinetic characterization of BA/BS transport by human MRP3 has recently been published [79].

2.1.6. MRP4/ABCC4

MRP4 is expressed on the sinusoidal membrane of hepatocytes [80]. MRP4 has been linked to hepatic bile salt transport as Mrp4 knockout mice showed an impaired cytoprotective response in obstructive cholestasis [81], and Mrp4 was significantly upregulated in Bsep knockout mice [82]. The upregulation of MRP4 was also shown in the inherited BSEP deficiency, PFIC-2 [83], as well as in patients with primary biliary cirrhosis (PBC) and

obstructive cholestasis [84]. The bile salt transport profile of human MRP4 was studied in V79 cells overexpressing MRP4 and was shown to be dependent on GSH cotransport [80]. Subsequent substrate specificity studies have shown the transport of cholate and several monovalent BS [85]. In a more recent study using vesicles derived from HEK293 cells overexpressing MRP4 or MRP3, the latter transported BS with significantly higher efficiency [79].

2.1.7. OSTα/β/SLC51A/B

OSTα/β is a complex that functions solely in a heterodimeric form [86]. It is broadly expressed in the body, especially in enterocytes, hepatocytes, and cholangiocytes [87]; therefore, it plays a major role in the enterohepatic and cholehepatic shunts of BA/BS [88,89]. Albeit OSTα/β is a bidirectional facilitative transporter, it is generally considered to provide an efflux function in hepatocytes. It is highly induced in chenodeoxycholate-treated sandwich-cultured human hepatocytes [88,90], and OSTβ (*SLC51B*) deficiency presents with features of cholestasis [91].

In mice, however, Ostα/β may also mediate the uptake of BA as knocking out Ostα provided protection in the obstructed liver. It appears that in the Ostα knockout mice, the compensatory upregulation of the Phase II enzymes (Ugt1a1 and Sult2a1) and basolateral efflux pumps Mrp3 and Mrp4 led to increased efflux and a subsequent increase in the renal elimination of the Phase II metabolites of BA/BS, thus lowering the hepatic BA/BS load [92]. The redundancy and intricate regulation of the basolateral efflux pathways shown in this study clearly demonstrate the importance of the basolateral efflux of BA/BS.

2.2. Phospholipid Translocases

A delicate balance of BA/BS, phospholipids, and cholesterol is key to the physicochemical stability of bile. The impaired homeostasis of phospholipids interferes with the excretion of BS and thus precipitates cholestasis. An important cooperation between the two major phospholipid translocases has been shown in a study where overexpression of MDR3 was toxic to HEK293T cells, and this toxicity was counteracted by the concomitant overexpression of the ATP8B1–CDC50A complex [93]. In ATP8A1 knockout mice, but not ATP8A1/MDR3 double knockouts, the BS-induced extraction of phospholipids and proteins from the canalicular membrane was observed [93].

2.2.1. MDR3/ABCB4

The multidrug resistance protein 3 (MDR3) is a phosphatidylcholine floppase. The carriers of functionally defective variants of MDR3 have altered phosphatidylcholine/BS ratios and may develop cholelithiasis, intrahepatic cholestasis in pregnancy, or PFIC-3 [94]. MDR3 has been shown to transport drugs [95]; this capability may explain its broad inhibitor specificity [96].

2.2.2. ATP8B1

ATP8B1 translocates phosphatidylserine from the outer leaflet to the inner leaflet. Consistent with its function as an aminophospholipid flippase, the treatment of Atp8b1-deficient rat hepatocytes with CDC resulted in focal canalicular membrane disruption and the luminal accumulation of NBD-phosphatidylserine [97]. An impaired ATP8B1 function in humans can cause PFIC-1, although in a recent genotype–phenotype correlation study, the severity of genotype was not found to be associated with disease history or prognosis [98]. Van Wessel et al. [98] suggested that the lack of association could be partly due to the indirect role of ATP8B1 in BS transport. It is also possible that mutations in proteins that mediate the trafficking of ATP8B1, but not mutations in ATP8B1 itself, explain the functional impairment observed [99]. Interestingly, to date, there are no known drug inhibitors of ATP8B1 [96].

2.3. Regulation of Bile Salt Transport in Hepatocytes

Hepatocytes maintain BA homeostasis by regulating the expression of rate-limiting enzymes that mediate BA synthesis and/or modulating the levels of BA transporters [100]. An abundance of BA is a major regulatory signal. Bile acids are agonists of the farnesoid X receptor (FXR), and intestinal BA reabsorption induces FXR-mediated expression and the subsequent secretion of FGF19, the human ortholog of rodent Fgf15, in the gut. The transcriptional effects of FGF19 are mediated by small heterodimer partners (SHPs).

Acting through FGF4R located in the sinusoidal membrane of hepatocytes, FGF19 represses the expression of CYP7A1, the rate-limiting enzyme of BA synthesis [101]. FXR is expressed in hepatocytes, too, and BA abundance suppresses both the de novo synthesis of BA via repression of CYP7A1 [102] and the uptake of BA/BS via the repression of NTCP and OATPs [103,104]. FXR also facilitates the removal of BA/BS from hepatocytes via the induction of the canalicular BS transporters BSEP [105] and MRP2 [106] as well as the induction of sinusoidal OSTα/β but not MRP4 [107]. Notably, lithocholic acid, the most toxic BA is an FXR antagonist and thus inhibits the removal of BS from hepatocytes [108]. The activity of FXR is regulated partly by SIRT1 (Sirtuin-1). SIRT1 potentiates the transcriptional activity of FXR at multiple levels partly by the deacetylation of FXR [109,110].

The other key regulatory nuclear hormone receptor is the pregnane X receptor (PXR) which induces the expression of sulfotransferase SULT2A1 which, in turn, extensively sulfates BA/BS [50]. PXR agonists also induce the expression of MRP2 [111] and MRP3 [112], and both are shown to transport sulfated BA/BS [77,113] and probably induce MRP4, too [114], which has also been suggested to transport sulfated BA [115].

It has been proposed that BA accumulation triggers adaptive and adverse responses [116]. Adaptive responses include (i) shifting the BA pool towards more soluble BA, such as conjugated BS over unconjugated BA, cholates over chenodeoxycholates, tauro-conjugated BS over glyco-conjugated BS, and sulfated BS, (ii) the suppression of BS uptake, and (iii) the induction of BS efflux [17,37,50]. Therefore, drugs which interfere with adaptive responses might be especially harmful.

Cholestatic, but not non-cholestatic, drugs have recently been shown to inhibit the amidation and sulfation of BA in HepaRG cells [117]. A 24 h treatment with the cholestatic drug cyclosporine A correlated with up to 84% inhibition of the expression of the conjugating enzyme CoA:amino acid N-acyltransferase (BAAT) [118]. The internalization of BSEP is another mechanism that decreases the canalicular clearance of BA/BS [119]. Although mechanistic studies of internalization have been published, drug-induced internalization has not been extensively studied.

2.4. Mechanism of Hepatotoxicity of Bile Acids and Bile Salts

Bile acid toxicity affects multiple organelles including the mitochondria, the endoplasmic reticulum (ER), and endosomes/lysosomes. Oxidative stress is one of the adverse responses inflicted on hepatocytes upon BA accumulation, and mitochondria are a major source of oxygen radicals [120]. Unlike non-hepatotoxic BS, hepatotoxic BS damage the inner mitochondrial membrane and precipitate mitochondrial permeability transition (MPT) [59]. BA/BS-induced apoptosis in rat hepatocytes was inhibited by MPT blockers and antioxidants [121]. Whereas it is generally accepted that hydrophobic BS elicit mitochondrial toxicity, the effect may depend on the choice of experimental system. The direct mitochondrial toxicity of BS has been observed in isolated human and rat liver mitochondria as well as HepG2 cells but not HepaRG cells [60,120,122,123]. In addition, in rat liver mitochondria UDC, TUDC and GUDC prevented MPT as assessed via monitoring mitochondrial swelling [122], whereas in HepG2 cells, TUDC failed to prevent CDC-induced apoptosis, and UDC even potentiated CDC-induced necrotic cell death [123]. This apparent discrepancy may partly be accounted for by inter-species differences, considering that CDC is the most abundant bile acid in humans but a minor component in rats [124].

Endoplasmic reticulum (ER) stress is also accompanied by the generation of reactive oxygen species (ROS) because the accumulation of misfolded proteins in the ER leads to

the leakage of Ca^{2+} which triggers ROS production [125,126]. Impaired lysosomal function upon accumulation of BA/BS has also been described. BS have been shown to impair autophagosome formation, and the BS-induced blockade of autophagy is mediated by FXR [127]. This may seem counterintuitive as FXR is generally thought to orchestrate a protective response, and autophagy is considered as cytoprotective. A potential interpretation is that excessive autophagy may be detrimental [128], and the effect of FXR is bimodal. This, however, needs to be experimentally confirmed. In addition to the cell-autonomous toxic effects, the overproduction of BA/BS at a tissue level triggers inflammation, with significant contributions from cholangiocytes and the immune cells of the liver [129].

3. In Vitro Test Systems for the Study of Bile Acid/Bile Salt Transport

Whereas native BA/BS are broadly considered the most relevant probes to be used in vitro, fluorescent substrates, BA analogs, and unrelated compounds alike have been employed in both vesicular and cellular assays [130–132].

The BA/BS uptake by NTCP, OATP1B1, and OATP1B3 has been studied routinely in cell lines overexpressing the respective recombinant uptake transporter [38,43]. Other options include using suspension hepatocytes, adherent hepatocyte cultures, or sandwich-cultured hepatocytes [133,134]. Early tests with OSTα/β were carried out in oocytes [135]. An MDCKII-based double transfectant system co-expressing ASBT and OSTα/β was used in a large inhibition screen of 1280 FDA-approved drugs [136]. More recent studies of OSTα/β inhibition have employed Flp-In-HEK293-based and MDCKII-based transfectants [89,137].

For efflux transporter studies, membrane vesicle assays are the method of choice. Vesicular transport studies on bile salt transport by all major transporters including BSEP, MRP2, MRP3, and MRP4 have been published [77,79,85,113,138,139]. Sandwich-cultured hepatocytes have also been used to investigate bile salt efflux [140]. Liver spheroids were found similarly suitable for the assessment of BA/BS transporter function using fluorescent BA as substrates and were leveraged to model drug-induced cholestasis [141,142]. Thanks to the disparate substrate recognition of BSEP and MRP2, the canalicular BSEP function can be studied in these holistic cellular models with reasonable specificity. In contrast, the basolateral efflux functions provided by MRP3, MRP4, and OSTα/β are difficult to differentiate unless using knockout or knockdown models.

Testing of Bile Salt Transport to Assess Cholestatic Liver Injury

It is generally accepted that interference with BS transport is a key causal factor in cholestasis. Major publications from both the industry and the International Transporter Consortium have recommended the testing of BSEP inhibition. The relevant papers with suggested cut-off values [143–149] are summarized in Table 1. Most studies employ a vesicular transport assay with taurocholate as a probe, albeit some cholestatic compounds have been shown to act via repression of BSEP expression, which can only be investigated in cellular models [150].

Table 1. Recommendations for the in vitro evaluation of cholestatic risk. Abbreviations: BEI, biliary excretion index; hMPCC, hepatocyte micropatterned co-culture; SCH, sandwich-cultured hepatocytes; TC, taurocholate; VT, vesicular transport.

Source	Morgan et al., 2013 [144]	Schadt et al., 2015 [143]	Riede et al., 2017 [146]	Yucha et al., 2017 [147]	Kenna et al., 2018 [145]	Aleo et al., 2020 [148]	Hafey et al., 2020 [149]
BSEP assay method	VT (probe: TC)	VT (probe: TC)	VT (probe: TC)	VT (probe: TC)	VT (probe: TC) or SCH (probe: TC)	VT (probe: TC)	VT (probe: TC)
Recommended DILI risk cut-off/score for BSEP	$C_{ss}/IC_{50} \geq 0.1$	$C_{plasma}/IC_{50} > 0.05$	$IC_{50}/C_{hep,inlet,u} < 26$: cholestasis common; $26 < IC_{50}/C_{hep,inlet,u} < 529$: cholestasis rare	$IC_{50} < 50$ µM	Drugs in discovery: $IC_{50} \leq 25$µM Drugs in discovery to Phase I–II: $C_{ss}/IC_{50} \geq 0.1$	$IC_{50}/C_{max,total}$: Score = 1 if >50–≤100); Score = 2 if >10–≤50); Score = 3 if >1–≤10); Score = 4 if ≤1);	$IC_{50} < 5$ µM
Recommendations for other transporter interaction assays	MRP2, MRP3, and MRP4 recommended				MRP2, MRP3, MRP4, OSTα/β, NTCP, OATP1B1, and OATP1B3 recommended in the case of clinically relevant BSEP inhibition		MRP2, MRP3, and MRP4 *not* recommended
Other recommended assays		Reactive metabolites; mitochondrial toxicity; cellular toxicity	In vitro $K_{p,uu}$		Reactive metabolites; mitochondrial toxicity; oxidative stress	Cytotoxicity; mitochondrial dysfunction (isolated mitochondria); mitochondrial dysfunction (cell)	Inhibition of TC transport in hMPCC: IC_{50} for clearance and BEI
Further considerations				MW > 600; cLogP > 3; dose > 100 mg/day		Physicochemical properties	

Studies referring to C_{ss}/IC_{50} values use 0.05 and 0.1 as cut-offs [143–145], whereas $IC_{50}/C_{hep,inlet,u} < 26$ (ca. $C_{hep,inlet,u}/IC_{50} > 0.04$) may also serve as an indicator [146]. For compounds in the discovery phase where the C_{plasma} values are still only predicted and may thus be unreliable, risk assessment can be based solely on IC_{50}, and different groups have suggested cut-off values of 25 [145] and 50 μM [147]. Aleo et al. have applied a complex scoring system, with the highest score (most severe category) assigned to compounds with $IC_{50}/C_{max,total} \leq 1$ and the safe zone for BSEP inhibition starting at $IC_{50}/C_{max,total} > 100$ [148]. In other studies, the utility of in vitro BSEP inhibition for predicting DILI has been confirmed without recommending a specific cut-off [96,108].

Whether in vitro inhibition assays for other bile acid and bile salt transporters may add value to DILI risk assessment is a matter of debate. Although the overall effect of the inhibition of influx and efflux transporters is challenging to quantify, initial integrative models have been published [151,152]. Inhibition of any of the MRPs (i.e., MRP2, MRP3, or MRP4) with a cut-off of $C_{ss}/IC_{50} \geq 0.1$ has been considered a significant contributor to cholestasis and was suggested to be included in decision-making [144]. Inhibition of the sinusoidal efflux functions MRP3, MRP4, and OSTα/β has also been acknowledged as significant in several studies [153,154]. Conversely, the inclusion of MRP2, MRP3, and MRP4 inhibition did not improve the predictability of DILI in a more recent study [149]. Meanwhile, the ITC paper recommended a broader panel that included OATP1B1, OATP1B3, and NTCP in addition to MRP2-4 as a follow-up to clinically relevant ($C_{ss}/IC_{50} \geq 0.1$) BSEP inhibition [145].

The genetic impairment of MDR3 function leads to PFIC-3, and the inhibition of MDR3 by antifungals has been linked to cholestasis [155,156]. The possible cholestatic effect of MDR3 inhibition was addressed in a large study that assayed 125 drugs on hepatocytes using d9-phosphatidyl-choline as a probe [148]. "Most-DILI concern compounds" inhibited MDR3 and BSEP with $IC_{50} < 20$ μM. However, whether the substrate transport was specific to MDR3 remained a concern [145], and testing the inhibition of transporters other than BSEP was found to have minimal beneficial impacts on the prediction of DILI [148].

Drugs may not only contribute to DILI by inhibiting bile salt efflux. Other mechanisms that may precipitate or aggravate hepatotoxicity include the blockade of FXR-mediated adaptive responses [157,158] or the inhibition of amidation and sulfation [117]. Hence, the mechanism of DILI is complex, and even the clinical presentation of cholestasis may be highly variable [17,159]. Other than bile salt transport inhibition, new testing paradigms encompass the mitochondrial toxicity, generation of ROS, cytotoxicity that may be related to the accumulation of bile salts, and formation of reactive metabolites. High doses (≥100 mg), lipophilicity (cLogP ≥ 3), and high molecular weights (>600) are further attributes that have been linked to hepatotoxic potential [17,147,148].

4. Conclusions

For compounds in the discovery stage, a BSEP $IC_{50} \leq 25$ μM threshold has been suggested [145]. In the presence of additional compound- and dose-related risk factors, such as MW > 600, cLogP > 3, and oral dose >100 mg, BSEP $IC_{50} \leq 50$ μM may be a reasonable cut-off [147,148]. For compounds in the discovery/clinical Phases I–II with predicted total $C_{ss,plasma}$/BSEP $IC_{50} \geq 0.1$, testing for the inhibition of other relevant hepatocyte bile acid transporters may be needed [145].

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: The authors gratefully appreciate the formatting, careful and critical reading, and meticulous correction of the paper by Noémi Szili.

Conflicts of Interest: Péter Krajcsi is a co-founder and consultant of Habilitas Ltd., Budapest, Hungary, a former employee of SOLVO Biotechnology, Budapest, Hungary, and a current employee of Targetex Ltd., Budapest, Hungary. Péter Tátrai is a current employee of Charles River Laboratories

Hungary (former SOLVO Biotechnology). None of the companies listed had any role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Alexander, S.P.H.; Kelly, E.; Mathie, A.; Peters, J.A.; Veale, E.L.; Armstrong, J.F.; Faccenda, E.; Harding, S.D.; Pawson, A.J.; Sharman, J.L.; et al. The Concise Guide to Pharmacology 2019/20: Transporters. *Br. J. Pharmacol.* **2019**, *176* (Suppl. 1), S397–S493. [CrossRef]
2. International Transporter Consortium; Giacomini, K.M.; Huang, S.M.; Tweedie, D.J.; Benet, L.Z.; Brouwer, K.L.; Chu, X.; Dahlin, A.; Evers, R.; Fischer, V. Membrane transporters in drug development. *Nat. Rev. Drug Discov.* **2010**, *9*, 215–236.
3. Chu, X.; Liao, M.; Shen, H.; Yoshida, K.; Zur, A.A.; Arya, V.; Galetin, A.; Giacomini, K.M.; Hanna, I.; Kusuhara, H.; et al. Clinical Probes and Endogenous Bi-omarkers as Substrates for Transporter Drug-Drug Interaction Evaluation: Perspectives from the International Transporter Consortium. *Clin. Pharmacol. Ther.* **2018**, *104*, 836–864. [CrossRef]
4. Shen, H.; Marathe, P. Endogenous Biomarkers to Assess Drug-Drug Interactions by Drug Transporters and Enzymes. *Curr. Drug Metab.* **2017**, *18*, 757–768. [CrossRef]
5. Rodrigues, D.; Rowland, A. From Endogenous Compounds as Biomarkers to Plasma-Derived Nanovesicles as Liquid Biopsy; Has the Golden Age of Translational Pharmacokinetics-Absorption, Distribution, Metabolism, Excretion-Drug-Drug Interaction Science Finally Arrived? *Clin. Pharmacol. Ther.* **2019**, *105*, 1407–1420. [CrossRef]
6. Tátrai, P.; Krajcsi, P. Prediction of Drug-Induced Hyperbilirubinemia by In Vitro Testing. *Pharmaceutics* **2020**, *12*, 755. [CrossRef]
7. Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation; U.S. Department of Health and Human Services; Food and Drug Administration Center for Drug Evaluation and Research (CDER); Center for Biologics Evaluation and Research (CBER). July 2009, Drug Safety, Docket Number: FDA-2008-D-0128. Available online: https://www.fda.gov/media/116737/download (accessed on 21 December 2022).
8. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Drug-induced liver injury. *J. Hepatol.* **2019**, *70*, 1222–1261. [CrossRef]
9. Mosedale, M.; Watkins, P.B. Drug-induced liver injury: Advances in mechanistic understanding that will inform risk manage-ment. *Clin. Pharmacol. Ther.* **2017**, *101*, 469–480. [CrossRef]
10. Senior, J.R. What is idiosyncratic hepatotoxicity? What is it not? *Hepatology* **2008**, *47*, 1813–1815. [CrossRef]
11. Bénichou, C. Criteria of drug-induced liver disorders: Report of an International Consensus Meeting. *J. Hepatol.* **1990**, *11*, 272–276. [CrossRef]
12. Björnsson, E.; Nordlinder, H.; Olsson, R. Clinical characteristics and prognostic markers in disulfiram-induced liver injury. *J. Hepatol.* **2006**, *44*, 791–797. [CrossRef]
13. Vinken, M.; Hengstler, J.G. Characterization of hepatocyte-based in vitro systems for reliable toxicity testing. *Arch. Toxicol.* **2018**, *92*, 2981–2986. [CrossRef]
14. Liu, Y.; Li, P.; Wang, F.; Liu, L.; Zhang, Y.; Liu, Y.; Shi, R. Comparison of diagnostic accuracy of 3 diagnostic criteria combined with refined pathological scoring system for drug-induced liver injury. *Medicine* **2020**, *99*, e22259. [CrossRef]
15. Poupon, R. Liver alkaline phosphatase: A missing link between choleresis and biliary inflammation. *Hepatology* **2015**, *61*, 2080–2090. [CrossRef]
16. Hertel, P.M.; Bull, L.N.; Thompson, R.J.; Goodrich, N.P.; Ye, W.; Magee, J.C.; Squires, R.H.; Bass, L.M.; Heubi, J.E.; Kim, G.E.; et al. Mutation Analysis and Disease Features at Presentation in a Multi-Center Cohort of Children with Monogenic Cholestasis. *J. Pediatr. Gastroenterol. Nutr.* **2021**, *73*, 169–177. [CrossRef]
17. Yang, K.; Köck, K.; Sedykh, A.; Tropsha, A.; Brouwer, K.L. An updated review on drug-induced cholestasis: Mechanisms and in-vestigation of physicochemical properties and pharmacokinetic parameters. *J. Pharm. Sci.* **2013**, *102*, 3037–3057. [CrossRef]
18. Wang, L.; Prasad, B.; Salphati, L.; Chu, X.; Gupta, A.; Hop, C.E.; Evers, R.; Unadkat, J.D. Interspecies Variability in Expression of Hepatobiliary Transporters across Human, Dog, Monkey, and Rat as Determined by Quantitative Proteomics. *Drug Metab. Dispos.* **2014**, *43*, 367–374. [CrossRef]
19. Kullak-Ublick, G.A.; Beuers, U.; Paumgartner, G. Hepatobiliary transport. *J. Hepatol.* **2000**, *32* (Suppl. 1), 3–18. [CrossRef]
20. Kullak-Ublick, G.A.; Stieger, B.; Meier, P.J. Enterohepatic bile salt transporters in normal physiology and liver disease. *Gastroenterology* **2004**, *126*, 322–342. [CrossRef]
21. Hagenbuch, B.; Meier, P.J. Molecular cloning, chromosomal localization, and functional characterization of a human liver Na+/bile acid cotransporter. *J. Clin. Investig.* **1994**, *93*, 1326–1331. [CrossRef] [PubMed]
22. Dong, Z.; Ekins, S.; Polli, J.E. A substrate pharmacophore for the human sodium taurocholate co-transporting polypeptide. *Int. J. Pharm.* **2015**, *478*, 88–95. [CrossRef]
23. Dong, Z.; Ekins, S.; Polli, J.E. Quantitative NTCP pharmacophore and lack of association between DILI and NTCP Inhibition. *Eur. J. Pharm. Sci.* **2015**, *66*, 1–9. [CrossRef]
24. Dawson, P.A.; Lan, T.; Rao, A. Bile acid transporters. *J. Lipid Res.* **2009**, *50*, 2340–2357. [CrossRef]
25. Stieger, B. The role of the sodium-taurocholate cotransporting polypeptide (NTCP) and of the bile salt export pump (BSEP) in physiology and pathophysiology of bile formation. *Handb. Exp. Pharmacol.* **2011**, *201*, 205–259.

26. Geyer, J.; Wilke, T.; Petzinger, E. The solute carrier family SLC10: More than a family of bile acid transporters regarding function and phylogenetic relationships. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2006**, *372*, 413–431. [CrossRef]
27. Greupink, R.; Dillen, L.; Monshouwer, M.; Huisman, M.T.; Russel, F.G. Interaction of fluvastatin with the liver-specific Na+-dependent taurocholate cotransporting polypeptide (NTCP). *Eur. J. Pharm. Sci.* **2011**, *44*, 487–496. [CrossRef]
28. Russell, L.E.; Zhou, Y.; Lauschke, V.M.; Kim, R.B. In Vitro Functional Characterization and in Silico Prediction of Rare Genetic Variation in the Bile Acid and Drug Transporter, Na^+-Taurocholate Cotransporting Polypeptide (NTCP, *SLC10A1*). *Mol. Pharm.* **2020**, *17*, 1170–1181. [CrossRef]
29. Pan, W.; Song, I.-S.; Shin, H.-J.; Kim, M.-H.; Choi, Y.-L.; Lim, S.-J.; Kim, W.-Y.; Lee, S.-S.; Shin, J.-G. Genetic polymorphisms in Na^+-taurocholate co-transporting polypeptide (NTCP) and ileal apical sodium-dependent bile acid transporter (ASBT) and ethnic comparisons of functional variants of NTCP among Asian populations. *Xenobiotica* **2011**, *41*, 501–510. [CrossRef]
30. Deng, M.; Mao, M.; Guo, L.; Chen, F.P.; Wen, W.R.; Song, Y.Z. Clinical and molecular study of a pediatric patient with sodium tau-rocholate cotransporting polypeptide deficiency. *Exp. Ther. Med.* **2016**, *12*, 3294–3300. [CrossRef]
31. Li, H.; Deng, M.; Guo, L.; Qiu, J.; Lin, G.; Long, X.; Xiao, X.; Song, Y. Clinical and molecular characterization of four patients with NTCP deficiency from two unrelated families harboring the novel SLC10A1 variant c.595A>C (p.Ser199Arg). *Mol. Med. Rep.* **2019**, *20*, 4915–4924. [CrossRef]
32. Hu, M.; Fok, B.S.; Wo, S.-K.; Lee, V.H.; Zuo, Z.; Tomlinson, B. Effect of common polymorphisms of the farnesoid X receptor and bile acid transporters on the pharmacokinetics of ursodeoxycholic acid. *Clin. Exp. Pharmacol. Physiol.* **2015**, *43*, 34–40. [CrossRef]
33. Lin, H.; Qiu, J.-W.; Rauf, Y.-M.; Lin, G.-Z.; Liu, R.; Deng, L.-J.; Deng, M.; Song, Y.-Z. Sodium Taurocholate Cotransporting Polypeptide (NTCP) Deficiency Hidden Behind Citrin Deficiency in Early Infancy: A Report of Three Cases. *Front. Genet.* **2019**, *10*, 1108. [CrossRef]
34. Vaz, F.M.; Huidekoper, H.H.; Paulusma, C.C. Extended Abstract: Deficiency of Sodium Taurocholate Cotransporting Polypeptide (SLC10A1): A New Inborn Error of Metabolism with an Attenuated Phenotype. *Dig. Dis.* **2017**, *35*, 259–260. [CrossRef]
35. Vaz, F.M.; Paulusma, C.; Huidekoper, H.; De Ru, M.; Lim, C.; Koster, J.; Ho-Mok, K.; Bootsma, A.H.; Groen, A.K.; Schaap, F.; et al. Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: Conjugated hypercholanemia without a clear clinical phenotype. *Hepatology* **2014**, *61*, 260–267. [CrossRef]
36. Mao, F.; Liu, T.; Hou, X.; Zhao, H.; He, W.; Li, C.; Jing, Z.; Sui, J.; Wang, F.; Liu, X.; et al. Increased sulfation of bile acids in mice and human subjects with sodium taurocholate cotransporting polypeptide deficiency. *J. Biol. Chem.* **2019**, *294*, 11853–11862. [CrossRef]
37. Humbert, L.; Maubert, M.A.; Wolf, C.; Duboc, H.; Mahé, M.; Farabos, D.; Seksik, P.; Mallet, J.M.; Trugnan, G.; Masliah, J.; et al. Bile acid profiling in human biological samples: Comparison of extraction procedures and application to normal and cholestatic patients. *J. Chromatogr. B* **2012**, *899*, 135–145. [CrossRef]
38. Jani, M.; Beéry, E.; Heslop, T.; Tóth, B.; Jagota, B.; Kis, E.; Park, B.K.; Krajcsi, P.; Weaver, R.J. Kinetic characterization of bile salt transport by human NTCP (SLC10A1). *Toxicol. Vitr.* **2018**, *46*, 189–193. [CrossRef]
39. Leslie, E.M.; Watkins, P.B.; Kim, R.B.; Brouwer, K.L. Differential inhibition of rat and human Na+-dependent taurocholate co-transporting polypeptide (NTCP/SLC10A1)by bosentan: A mechanism for species differences in hepatotoxicity. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 1170–1178. [CrossRef]
40. Sommer, J.; Dorn, C.; Gäbele, E.; Bataille, F.; Freese, K.; Seitz, T.; Thasler, W.E.; Büttner, R.; Weiskirchen, R.; Bosserhoff, A.; et al. Four-And-A-Half LIM-Domain Protein 2 (FHL2) Deficiency Aggravates Cholestatic Liver Injury. *Cells* **2020**, *9*, 248. [CrossRef]
41. Stieger, B.; Hagenbuch, B. Organic anion-transporting polypeptides. *Curr. Top. Membr.* **2014**, *73*, 205–232.
42. Mahagita, C.; Grassl, S.M.; Piyachaturawat, P.; Ballatori, N. Human organic anion transporter 1B1 and 1B3 function as bidirectional carriers and do not mediate GSH-bile acid cotransport. *Am. J. Physiol. Liver Physiol.* **2007**, *293*, G271–G278. [CrossRef]
43. Toth, B.; Jani, M.; Beéry, E.; Heslop, T.; Bayliss, M.; Kitteringham, N.R.; Park, B.K.; Weaver, R.J.; Krajcsi, P. Human OATP1B1 (SLCO1B1) transports sulfated bile acids and bile salts with particular efficiency. *Toxicol. Vitr.* **2018**, *52*, 189–194. [CrossRef]
44. Malagnino, V.; Hussner, J.; Issa, A.; Midzic, A.; zu Schwabedissen, H.M.; Zu Schwabedissen, H.E.M. OATP1B3-1B7, a novel organic anion transporting polypeptide, is modulated by FXR ligands and transports bile acids. *Am. J. Physiol. Liver Physiol.* **2019**, *317*, G751–G762. [CrossRef]
45. Takehara, I.; Terashima, H.; Nakayama, T.; Yoshikado, T.; Yoshida, M.; Furihata, K.; Watanabe, N.; Maeda, K.; Ando, O.; Sugiyama, Y.; et al. Investigation of Glycochenodeoxycholate Sulfate and Chenodeoxycholate Glucuronide as Surrogate Endogenous Probes for Drug Interaction Studies of OATP1B1 and OATP1B3 in Healthy Japanese Volunteers. *Pharm. Res.* **2017**, *34*, 1601–1614. [CrossRef]
46. Suga, T.; Yamaguchi, H.; Sato, T.; Maekawa, M.; Goto, J.; Mano, N. Preference of Conjugated Bile Acids over Unconjugated Bile Acids as Substrates for OATP1B1 and OATP1B3. *PLoS ONE* **2017**, *12*, e0169719. [CrossRef]
47. Zhou, D.; Kong, L.; Jiang, Y.; Wang, C.; Ni, Y.; Wang, Y.; Zhang, H.; Ruan, J. UGT-dependent regioselective glucuronidation of ur-sodeoxycholic acid and obeticholic acid and selective transport of the consequent acyl glucuronides by OATP1B1 and 1B3. *Chem. Biol. Interact.* **2019**, *310*, 108745. [CrossRef]
48. Yee, S.; Giacomini, M.; Hsueh, C.-H.; Weitz, D.; Liang, X.; Goswami, S.; Kinchen, J.; Coelho, A.; Zur, A.; Mertsch, K.; et al. Metabolomic and Genome-wide Association Studies Reveal Potential Endogenous Biomarkers for OATP1B1. *Clin. Pharmacol. Ther.* **2016**, *100*, 524–536. [CrossRef]

49. Rago, B.; Tierney, B.; Rodrigues, A.D.; Holliman, C.L.; Ramanathan, R. A multiplex HRMS assay for quantifying selected human plasma bile acids as candidate OATP biomarkers. *Bioanalysis* **2018**, *10*, 645–657. [CrossRef]
50. Rodrigues, A.D.; Lai, Y.; Cvijic, M.E.; Elkin, L.L.; Zvyaga, T.; Soars, M.G. Drug-Induced Perturbations of the Bile Acid Pool, Cholestasis, and Hepatotoxicity: Mechanistic Considerations beyond the Direct Inhibition of the Bile Salt Export Pump. *Drug Metab. Dispos.* **2013**, *42*, 566–574. [CrossRef]
51. Xiang, X.; Han, Y.; Neuvonen, M.; Pasanen, M.K.; Kalliokoski, A.; Backman, J.T.; Laitila, J.; Neuvonen, P.J.; Niemi, M. Effect of SLCO1B1 polymorphism on the plasma concentrations of bile acids and bile acid synthesis marker in humans. *Pharmacogenetics Genom.* **2009**, *19*, 447–457. [CrossRef]
52. Xiang, X.; Backman, J.T.; Neuvonen, P.J.; Niemi, M. Gender, but not CYP7A1 or SLCO1B1 Polymorphism, Affects the Fasting Plasma Concentrations of Bile Acids in Human Beings. *Basic Clin. Pharmacol. Toxicol.* **2011**, *110*, 245–252. [CrossRef]
53. van de Steeg, E.; Wagenaar, E.; van der Kruijssen, C.M.; Burggraaff, J.E.; de Waart, D.R.; Elferink, R.P.; Kenworthy, K.E.; Schinkel, A.H. Organic anion transporting polypeptide 1a/1b-knockout mice provide insights into hepatic handling of bilirubin, bile acids, and drugs. *J. Clin. Investig.* **2010**, *120*, 2942–2952. [CrossRef]
54. Slijepcevic, D.; Kaufman, C.; Wichers, C.G.; Gilglioni, E.H.; Lempp, F.A.; Duijst, S.; de Waart, D.R.; Elferink, R.P.O.; Mier, W.; Stieger, B.; et al. Impaired uptake of conjugated bile acids and hepatitis b virus pres1-binding in Na^+-taurocholate cotransporting polypeptide knockout mice. *Hepatology* **2015**, *62*, 207–219. [CrossRef]
55. Wang, L.; Soroka, C.J.; Boyer, J.L. The role of bile salt export pump mutations in progressive familial intrahepatic cholestasis type II. *J. Clin. Investig.* **2002**, *110*, 965–972. [CrossRef]
56. Wang, R.; Lam, P.; Liu, L.; Forrest, D.; Yousef, I.M.; Mignault, D.; Phillips, M.J.; Ling, V. Severe cholestasis induced by cholic acid feeding in knockout mice of sister of P-glycoprotein. *Hepatology* **2003**, *38*, 1489–1499. [CrossRef] [PubMed]
57. Wang, R.; Chen, H.-L.; Liu, L.; Sheps, J.A.; Phillips, M.J.; Ling, V. Compensatory role of P-glycoproteins in knockout mice lacking the bile salt export pump. *Hepatology* **2009**, *50*, 948–956. [CrossRef]
58. Jemnitz, K.; Veres, Z.; Vereczkey, L. Contribution of High Basolateral Bile Salt Efflux to the Lack of Hepatotoxicity in Rat in Response to Drugs Inducing Cholestasis in Human. *Toxicol. Sci.* **2010**, *115*, 80–88. [CrossRef]
59. Schulz, S.; Schmitt, S.; Wimmer, R.; Aichler, M.; Eisenhofer, S.; Lichtmannegger, J.; Eberhagen, C.; Artmann, R.; Tookos, F.; Walch, A.; et al. Progressive stages of mitochondrial destruction caused by cell toxic bile salts. *Biochim. Biophys. Acta (BBA)—Biomembr.* **2013**, *1828*, 2121–2133. [CrossRef]
60. Penman, S.L.; Sharma, P.; Aerts, H.; Park, B.K.; Weaver, R.J.; Chadwick, A.E. Differential toxic effects of bile acid mixtures in isolated mitochondria and physiologically relevant HepaRG cells. *Toxicol. Vitr.* **2019**, *61*, 104595. [CrossRef]
61. Jansen, P.L.M.; Peters, W.H.; Lamers, W.H. Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport. *Hepatology* **1985**, *5*, 573–579. [CrossRef]
62. Böhme, M.; Müller, M.; Leier, I.; Jedlitschky, G.; Keppler, D. Cholestasis caused by inhibition of the adenosine triphos-phate-dependent bile salt transport in rat liver. *Gastroenterology* **1994**, *107*, 255–265. [CrossRef] [PubMed]
63. Kartenbeck, J.; Leuschner, U.; Mayer, R.; Keppler, D. Absence of the canalicular isoform of theMRP gene-encoded conjugate export pump from the hepatocytes in Dubin-Johnson syndrome. *Hepatology* **1996**, *23*, 1061–1066. [CrossRef] [PubMed]
64. Iyanagi, T.; Emi, Y.; Ikushiro, S.-I. Biochemical and molecular aspects of genetic disorders of bilirubin metabolism. *Biochim. Biophys. Acta (BBA)—Mol. Basis Dis.* **1998**, *1407*, 173–184. [CrossRef]
65. Rost, D.; Kloeters-Plachky, P.; Stiehl, A. Retrieval of the rat canalicular conjugate export pump Mrp2 is associated with a rear-rangement of actin filaments and radixin in bile salt-induced cholestasis. *Eur. J. Med. Res.* **2008**, *13*, 314–318.
66. Keppler, D. Cholestasis and the Role of Basolateral Efflux Pumps. *Z. Gastroenterol.* **2011**, *49*, 1553–1557. [CrossRef] [PubMed]
67. Geier, A.; Dietrich, C.G.; Grote, T.; Beuers, U.; Prüfer, T.; Fraunberger, P.; Matern, S.; Gartung, C.; Gerbes, A.L.; Bilzer, M. Characterization of organic anion transporter regulation, glutathione metabolism and bile formation in the obese Zucker rat. *J. Hepatol.* **2005**, *43*, 1021–1030. [CrossRef] [PubMed]
68. Bodó, A.; Bakos, E.; Szeri, F.; Váradi, A.; Sarkadi, B. Differential Modulation of the Human Liver Conjugate Transporters MRP2 and MRP3 by Bile Acids and Organic Anions. *J. Biol. Chem.* **2003**, *278*, 23529–23537. [CrossRef]
69. Gerk, P.M.; Li, W.; Megaraj, V.; Vore, M. Human multidrug resistance protein 2 transports the therapeutic bile salt tauroursode-oxycholate. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 893–899. [CrossRef]
70. Stieger, B.; Fattinger, K.; Madon, J.; Kullak-Ublick, G.A.; Meier, P.J. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. *Gastroenterology* **2000**, *118*, 422–430. [CrossRef]
71. König, J.; Rost, D.; Cui, Y.; Keppler, D. Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. *Hepatology* **1999**, *29*, 1156–1163. [CrossRef]
72. Soroka, C.J.; Lee, J.M.; Azzaroli, F.; Boyer, J.L. Cellular localization and up-regulation of multidrug resistance–associated protein 3 in hepatocytes and cholangiocytes during obstructive cholestasis in rat liver. *Hepatology* **2001**, *33*, 783–791. [CrossRef] [PubMed]
73. Akita, H.; Suzuki, H.; Sugiyama, Y. Sinusoidal efflux of taurocholate correlates with the hepatic expression level of Mrp3. *Biochem. Biophys. Res. Commun.* **2002**, *299*, 681–687. [CrossRef] [PubMed]
74. Zelcer, N.; van de Wetering, K.; de Waart, R.; Scheffer, G.L.; Marschall, H.-U.; Wielinga, P.R.; Kuil, A.; Kunne, C.; Smith, A.; van der Valk, M.; et al. Mice lacking Mrp3 (Abcc3) have normal bile salt transport, but altered hepatic transport of endogenous glucuronides. *J. Hepatol.* **2006**, *44*, 768–775. [CrossRef] [PubMed]

75. Belinsky, M.G.; Dawson, P.A.; Shchaveleva, I.; Bain, L.J.; Wang, R.; Ling, V.; Chen, Z.-S.; Grinberg, A.; Westphal, H.; Klein-Szanto, A.; et al. Analysis of the In Vivo Functions of Mrp3. *Mol. Pharmacol.* **2005**, *68*, 160–168. [CrossRef] [PubMed]
76. Chai, J.; He, Y.; Cai, S.Y.; Jiang, Z.; Wang, H.; Li, Q.; Chen, L.; Peng, Z.; He, X.; Wu, X. Elevated hepatic multidrug resistance-associated protein 3/ATP-binding cassette subfamily C3 expression in human obstructive cholestasis is mediated through tumor necrosis factor alpha and c-Jun NH2-terminal kinase/stress-activated protein kinase-signaling pathway. *Hepatology* **2012**, *55*, 1485–1494.
77. Akita, H.; Suzuki, H.; Hirohashi, T.; Takikawa, H.; Sugiyama, Y. Transport activity of human MRP3 expressed in Sf9 cells: Com-parative studies with rat MRP3. *Pharm. Res.* **2002**, *19*, 34–41. [CrossRef]
78. Hirohashi, T.; Suzuki, H.; Takikawa, H.; Sugiyama, Y. ATP-dependent Transport of Bile Salts by Rat Multidrug Resistance-associated Protein 3 (Mrp3). *J. Biol. Chem.* **2000**, *275*, 2905–2910. [CrossRef]
79. Chothe, P.P.; Pemberton, R.; Hariparsad, N. Function and Expression of Bile Salt Export Pump in Suspension Human Hepatocytes. *Drug Metab. Dispos.* **2021**, *49*, 314–321. [CrossRef]
80. Rius, M.; Nies, A.T.; Hummel-Eisenbeiss, J.; Jedlitschky, G.; Keppler, D. Cotransport of reduced glutathione with bile salts by MRP4 (ABCC4) localized to the basolateral hepatocyte membrane. *Hepatology* **2003**, *38*, 374–384. [CrossRef]
81. Mennone, A.; Soroka, C.J.; Cai, S.Y.; Harry, K.; Adachi, M.; Hagey, L.; Schuetz, J.D.; Boyer, J.L. Mrp4-/- mice have an impaired cyto-protective response in obstructive cholestasis. *Hepatology* **2006**, *43*, 1013–1021. [CrossRef]
82. Wang, R.; Liu, L.; Sheps, J.A.; Forrest, D.; Hofmann, A.F.; Hagey, L.R.; Ling, V. Defective canalicular transport and toxicity of dietary ursodeoxycholic acid in the abcb11-/- mouse: Transport and gene expression studies. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2013**, *305*, G286–G294. [CrossRef]
83. Keitel, V.; Burdelski, M.; Warskulat, U.; Kühlkamp, T.; Keppler, D.; Häussinger, D.; Kubitz, R. Expression and localization of hepatobiliary transport proteins in progressive familial intrahepatic cholestasis. *Hepatology* **2005**, *41*, 1160–1172. [CrossRef]
84. Chai, J.; Luo, D.; Wu, X.; Wang, H.; He, Y.; Li, Q.; Zhang, Y.; Chen, L.; Peng, Z.-H.; Xiao, T.; et al. Changes of Organic Anion Transporter MRP4 and Related Nuclear Receptors in Human Obstructive Cholestasis. *J. Gastrointest. Surg.* **2011**, *15*, 996–1004. [CrossRef]
85. Rius, M.; Hummel-Eisenbeiss, J.; Hofmann, A.F.; Keppler, D. Substrate specificity of human ABCC4 (MRP4)-mediated cotransport of bile acids and reduced glutathione. *Am. J. Physiol. Liver Physiol.* **2006**, *290*, G640–G649. [CrossRef]
86. Beaudoin, J.J.; Brouwer, K.L.R.; Malinen, M.M. Novel insights into the organic solute transporter alpha/beta, OSTα/β: From the bench to the bedside. *Pharmacol. Ther.* **2020**, *211*, 107542. [CrossRef]
87. Ballatori, N.; Fang, F.; Christian, W.V.; Li, N.; Hammond, C.L. Ostalpha-Ostbeta is required for bile acid and conjugated steroid disposition in the intestine, kidney, and liver. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2008**, *295*, G179–G186. [CrossRef]
88. Beaudoin, J.J.; Bezençon, J.; Sjöstedt, N.; Fallon, J.K.; Brouwer, K.L.R. Role of Organic Solute Transporter Alpha/Beta in Hepatotoxic Bile Acid Transport and Drug Interactions. *Toxicol. Sci.* **2020**, *176*, 34–45. [CrossRef]
89. Suga, T.; Yamaguchi, H.; Ogura, J.; Mano, N. Characterization of conjugated and unconjugated bile acid transport via human organic solute transporter α/β. *Biochim. Biophys. Acta (BBA)—Biomembr.* **2019**, *1861*, 1023–1029. [CrossRef]
90. Jackson, J.P.; Freeman, K.M.; Friley, W.W.; St Claire, I.I.I.R.L.; Black, C.; Brouwer, K.R. Basolateral Efflux Transporters: A Potentially Important Pathway for the Prevention of Cholestatic Hepatotoxicity. *Appl. In Vitro Toxicol.* **2016**, *2*, 207–216. [CrossRef]
91. Sultan, M.; Rao, A.; Elpeleg, O.; Vaz, F.M.; Abu Libdeh, B.Y.; Karpen, S.J.; Dawson, P.A. Organic solute transporter-β (*SLC51B*) deficiency in two brothers with congenital diarrhea and features of cholestasis. *Hepatology* **2018**, *68*, 590–598. [CrossRef]
92. Soroka, C.J.; Mennone, A.; Hagey, L.R.; Ballatori, N.; Boyer, J.L. Mouse organic solute transporter α deficiency enhances renal excretion of bile acids and attenuates cholestasis. *Hepatology* **2009**, *51*, 181–190. [CrossRef] [PubMed]
93. Groen, A.; Romero, M.R.; Kunne, C.; Hoosdally, S.J.; Dixon, P.H.; Wooding, C.; Williamson, C.; Seppen, J.; Van Den Oever, K.; Mok, K.S.; et al. Complementary Functions of the Flippase ATP8B1 and the Floppase ABCB4 in Maintaining Canalicular Membrane Integrity. *Gastroenterology* **2011**, *141*, 1927–1937.e4. [CrossRef]
94. Aleo, M.D.; Shah, F.; He, K.; Bonin, P.D.; Rodrigues, A.D. Evaluating the Role of Multidrug Resistance Protein 3 (MDR3) Inhibition in Predicting Drug-Induced Liver Injury Using 125 Pharmaceuticals. *Chem. Res. Toxicol.* **2017**, *30*, 1219–1229. [CrossRef]
95. Smith, A.J.; van Helvoort, A.; van Meer, G.; Szabó, K.; Welker, E.; Szakács, G.; Váradi, A.; Sarkadi, B.; Borst, P. MDR3 P-glycoprotein, a Phosphatidylcholine Translocase, Transports Several Cytotoxic Drugs and Directly Interacts with Drugs as Judged by Interference with Nucleotide Trapping. *J. Biol. Chem.* **2000**, *275*, 23530–23539. [CrossRef]
96. Cai, S.; Gautam, S.; Nguyen, T.; Soroka, C.J.; Rahner, C.; Boyer, J.L. ATP8B1 Deficiency Disrupts the Bile Canalicular Membrane Bilayer Structure in Hepatocytes, But FXR Expression and Activity Are Maintained. *Gastroenterology* **2009**, *136*, 1060–1069.e4. [CrossRef]
97. van Wessel, D.B.E.; Thompson, R.J.; Gonzales, E.; Jankowska, I.; Shneider, B.L.; Sokal, E.; Grammatikopoulos, T.; Kadaristiana, A.; Jacquemin, E.; Spraul, A.; et al. Impact of Genotype, Serum Bile Acids, and Surgical Biliary Diversion on Native Liver Survival in FIC1 Deficiency. *Hepatology* **2021**, *74*, 892–906. [CrossRef]
98. Li, Q.; Sun, Y.; van Ijzendoorn, S.C.D. A Link between Intrahepatic Cholestasis and Genetic Variations in Intracellular Trafficking Regulators. *Biology* **2021**, *10*, 119. [CrossRef]
99. Wagner, M.; Zollner, G.; Trauner, M. New molecular insights into the mechanisms of cholestasis. *J. Hepatol.* **2009**, *51*, 565–580. [CrossRef]

100. Inagaki, T.; Choi, M.; Moschetta, A.; Peng, L.; Cummins, C.L.; McDonald, J.G.; Luo, G.; Jones, S.A.; Goodwin, B.; Richardson, J.A.; et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab.* **2005**, *2*, 217–225. [CrossRef]
101. Goodwin, B.; Jones, S.A.; Price, R.R.; Watson, M.A.; McKee, D.D.; Moore, L.B.; Galardi, C.; Wilson, J.G.; Lewis, M.C.; Roth, M.E.; et al. A Regulatory Cascade of the Nuclear Receptors FXR, SHP-1, and LRH-1 Represses Bile Acid Biosynthesis. *Mol. Cell* **2000**, *6*, 517–526. [CrossRef]
102. Gartung, C.; Ananthanarayanan, M.; Rahman, M.A.; Schuele, S.; Nundy, S.; Soroka, C.J.; Stolz, A.; Suchy, F.J.; Boyer, J.L. Down-regulation of expression and function of the rat liver Na+/bile acid cotransporter in extrahepatic cholestasis. *Gastroenterology* **1996**, *110*, 199–209. [CrossRef]
103. Jung, D.; Elferink, M.G.L.; Stellaard, F.; Groothuis, G.M.M. Analysis of bile acid-induced regulation of FXR target genes in human liver slices. *Liver Int.* **2007**, *27*, 137–144. [CrossRef]
104. Ananthanarayanan, M.; Balasubramanian, N.; Makishima, M.; Mangelsdorf, D.J.; Suchy, F.J. Human Bile Salt Export Pump Promoter Is Transactivated by the Farnesoid X Receptor/Bile Acid Receptor. *J. Biol. Chem.* **2001**, *276*, 28857–28865. [CrossRef]
105. Kast, H.R.; Goodwin, B.; Tarr, P.T.; Jones, S.A.; Anisfeld, A.M.; Stoltz, C.M.; Tontonoz, P.; Kliewer, S.; Willson, T.M.; Edwards, P.A. Regulation of Multidrug Resistance-associated Protein 2 (ABCC2) by the Nuclear Receptors Pregnane X Receptor, Farnesoid X-activated Receptor, and Constitutive Androstane Receptor. *J. Biol. Chem.* **2002**, *277*, 2908–2915. [CrossRef]
106. Renga, B.; Migliorati, M.; Mencarelli, A.; Cipriani, S.; D'Amore, C.; Distrutti, E.; Fiorucci, S. Farnesoid X receptor suppresses constitutive androstane receptor activity at the multidrug resistance protein-4 promoter. *Biochim. Biophys. Acta (BBA)—Gene Regul. Mech.* **2011**, *1809*, 157–165. [CrossRef]
107. Garzel, B.; Zhang, L.; Huang, S.-M.; Wang, H. A Change in Bile Flow: Looking Beyond Transporter Inhibition in the Development of Drug-induced Cholestasis. *Curr. Drug Metab.* **2019**, *20*, 621–632. [CrossRef]
108. Purushotham, A.; Xu, Q.; Lu, J.; Foley, J.F.; Yan, X.; Kim, D.-H.; Kemper, J.K.; Li, X. Hepatic Deletion of SIRT1 Decreases Hepatocyte Nuclear Factor 1α/Farnesoid X Receptor Signaling and Induces Formation of Cholesterol Gallstones in Mice. *Mol. Cell. Biol.* **2012**, *32*, 1226–1236. [CrossRef]
109. Kulkarni, S.R.; Soroka, C.J.; Hagey, L.R.; Boyer, J.L. Sirtuin 1 activation alleviates cholestatic liver injury in a cholic acid-fed mouse model of cholestasis. *Hepatology* **2016**, *64*, 2151–2164. [CrossRef]
110. Kauffmann, H.-M.; Pfannschmidt, S.; Zöller, H.; Benz, A.; Vorderstemann, B.; I Webster, J.; Schrenk, D. Influence of redox-active compounds and PXR-activators on human MRP1 and MRP2 gene expression. *Toxicology* **2001**, *171*, 137–146. [CrossRef]
111. Teng, S.; Piquette-Miller, M. Hepatoprotective role of PXR activation and MRP3 in cholic acid-induced cholestasis. *J. Cereb. Blood Flow Metab.* **2007**, *151*, 367–376. [CrossRef]
112. Akita, H.; Suzuki, H.; Ito, K.; Kinoshita, S.; Sato, N.; Takikawa, H.; Sugiyama, Y. Characterization of bile acid transport mediated by multidrug resistance associated protein 2 and bile salt export pump. *Biochim. Biophys. Acta (BBA)—Biomembr.* **2001**, *1511*, 7–16. [CrossRef]
113. Dolezelova, E.; Prasnicka, A.; Cermanova, J.; Carazo, A.; Hyrsova, L.; Hroch, M.; Mokry, J.; Adamcova, M.; Mrkvicova, A.; Pavek, P.; et al. Resveratrol modifies biliary secretion of cholephilic compounds in sham-operated and cholestatic rats. *World J. Gastroenterol.* **2017**, *23*, 7678–7692. [CrossRef]
114. Zelcer, N.; Reid, G.; Wielinga, P.; Kuil, A.; Van Der Heijden, I.; Schuetz, J.D.; Borst, P. Steroid and bile acid conjugates are substrates of human multidrug-resistance protein (MRP) 4 (ATP-binding cassette C4). *Biochem. J.* **2003**, *371*, 361–367. [CrossRef]
115. Vinken, M. In vitro prediction of drug-induced cholestatic liver injury: A challenge for the toxicologist. *Arch. Toxicol.* **2018**, *92*, 1909–1912. [CrossRef]
116. Burban, A.; Sharanek, A.; Humbert, L.; Eguether, T.; Guguen-Guillouzo, C.; Rainteau, M.; Guillouzo, A. Predictive Value of Cellular Accumulation of Hydrophobic Bile Acids As a Marker of Cholestatic Drug Potential. *Toxicol. Sci.* **2019**, *168*, 474–485. [CrossRef]
117. Sharanek, A.; Burban, A.; Humbert, L.; Azzi, P.B.-E.; Felix-Gomes, N.; Rainteau, D.; Guillouzo, A. Cellular Accumulation and Toxic Effects of Bile Acids in Cyclosporine A-Treated HepaRG Hepatocytes. *Toxicol. Sci.* **2015**, *147*, 573–587. [CrossRef]
118. Román, I.D.; Fernández-Moreno, M.D.; Fueyo, J.A.; Roma, M.G.; Coleman, R. Cyclosporin A induced internalization of the bile salt export pump in isolated rat hepatocyte couplets. *Toxicol. Sci.* **2003**, *71*, 276–281. [CrossRef]
119. Sokol, R.J.; Dahl, R.; Devereaux, M.W.; Yerushalmi, B.; E Kobak, G.; Gumpricht, E. Human Hepatic Mitochondria Generate Reactive Oxygen Species and Undergo the Permeability Transition in Response to Hydrophobic Bile Acids. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *41*, 235–243. [CrossRef]
120. Yerushalmi, B.; Dahl, R.; Devereaux, M.W.; Gumpricht, E.; Sokol, R.J. Bile acid-induced rat hepatocyte apoptosis is inhibited by antioxidants and blockers of the mitochondrial permeability transition. *Hepatology* **2001**, *33*, 616–626. [CrossRef]
121. Gores, G.J.; Miyoshi, H.; Botla, R.; Aguilar, H.I.; Bronk, S.F. Induction of the mitochondrial permeability transition as a mechanism of liver injury during cholestasis: A potential role for mitochondrial proteases. *Biochim. Biophys. Acta* **1998**, *1366*, 167–175. [CrossRef]
122. Rolo, A.P.; Palmeira, C.M.; Holy, J.M.; Wallace, K.B. Role of Mitochondrial Dysfunction in Combined Bile Acid-Induced Cytotoxicity: The Switch Between Apoptosis and Necrosis. *Toxicol. Sci.* **2004**, *79*, 196–204. [CrossRef]
123. Setchell, K.D.; Rodrigues, C.; Clerici, C.; Solinas, A.; Morelli, A.; Gartung, C.; Boyer, J. Bile acid concentrations in human and rat liver tissue and in hepatocyte nuclei. *Gastroenterology* **1997**, *112*, 226–235. [CrossRef]

124. Burban, A.; Sharanek, A.; Guguen-Guillouzo, C.; Guillouzo, A. Endoplasmic reticulum stress precedes oxidative stress in antibi-otic-induced cholestasis and cytotoxicity in human hepatocytes. *Free Radic. Biol. Med.* **2018**, *115*, 166–178. [CrossRef]
125. van der Vlies, D.; Makkinje, M.; Jansens, A.; Braakman, I.; Verkleij, A.J.; Wirtz, K.W.; Post, J.A. Oxidation of ER resident proteins upon oxidative stress: Effects of altering cellular redox/antioxidant status and implications for protein maturation. *Antioxid. Redox Signal.* **2003**, *5*, 381–387. [CrossRef]
126. Kim, S.; Han, S.Y.; Yu, K.S.; Han, D.; Ahn, H.-J.; Jo, J.-E.; Kim, J.-H.; Shin, J.; Park, H.-W. Impaired autophagy promotes bile acid-induced hepatic injury and accumulation of ubiquitinated proteins. *Biochem. Biophys. Res. Commun.* **2018**, *495*, 1541–1547. [CrossRef]
127. Nah, J.; Zhai, P.; Huang, C.-Y.; Fernández, F.; Mareedu, S.; Levine, B.; Sadoshima, J. Upregulation of Rubicon promotes autosis during myocardial ischemia/reperfusion injury. *J. Clin. Investig.* **2020**, *130*, 2978–2991. [CrossRef]
128. Li, M.; Cai, S.-Y.; Boyer, J.L. Mechanisms of bile acid mediated inflammation in the liver. *Mol. Asp. Med.* **2017**, *56*, 45–53. [CrossRef]
129. Mita, S.; Suzuki, H.; Akita, H.; Hayashi, H.; Onuki, R.; Hofmann, A.F.; Sugiyama, Y. Inhibition of bile acid transport across Na+/taurocholate cotransporting polypeptide (SLC10A1) and bile salt export pump (ABCB 11)-coexpressing LLC-PK1 cells by cholestasis-inducing drugs. *Drug Metab. Dispos.* **2006**, *34*, 1575–1581. [CrossRef]
130. Heredi-Szabo, K.; Kis, E.; Molnar, E.; Gyorfi, A.; Krajcsi, P. Characterization of 5(6)-carboxy-2′,7′-dichlorofluorescein transport by MRP2 and utilization of this substrate as a fluorescent surrogate for LTC4. *J. Biomol. Screen.* **2008**, *13*, 295–301. [CrossRef]
131. Brecklinghaus, T.; Albrecht, W.; Kappenberg, F.; Duda, J.; Vartak, N.; Edlund, K.; Marchan, R.; Ghallab, A.; Cadenas, C.; Günther, G.; et al. The hepatocyte export carrier inhibition assay improves the separation of hepatotoxic from non-hepatotoxic compounds. *Chem. Biol. Interact.* **2022**, *351*, 109728. [CrossRef]
132. De Bruyn, T.; Ye, Z.W.; Peeters, A.; Sahi, J.; Baes, M.; Augustijns, P.F.; Annaert, P.P. Determination of OATP-, NTCP- and OCT-mediated substrate uptake activities in individual and pooled batches of cryopreserved human hepatocytes. *Eur. J. Pharm. Sci.* **2011**, *43*, 297–307. [CrossRef] [PubMed]
133. Guo, C.; Yang, K.; Brouwer, K.R.; St Claire, R.L., 3rd; Brouwer, K.L. Prediction of Altered Bile Acid Disposition Due to Inhibition of Multiple Transporters: An Integrated Approach Using Sandwich-Cultured Hepatocytes, Mechanistic Modeling, and Simula-tion. *J. Pharmacol. Exp. Ther.* **2016**, *358*, 324–333. [CrossRef] [PubMed]
134. Seward, D.J.; Koh, A.S.; Boyer, J.L.; Ballatori, N.; Loris, R.; Marianovsky, I.; Lah, J.; Laeremans, T.; Engelberg-Kulka, H.; Glaser, G.; et al. Functional Complementation between a Novel Mammalian Polygenic Transport Complex and an Evolutionarily Ancient Organic Solute Transporter, OSTα-OSTβ. *J. Biol. Chem.* **2003**, *278*, 27473–27482. [CrossRef]
135. van de Wiel, S.M.W.; de Waart, D.R.; Oude Elferink, R.P.J.; van de Graaf, S.F.J. Intestinal Farnesoid X Receptor Activation by Phar-macologic Inhibition of the Organic Solute Transporter α-β. *Cell Mol. Gastroenterol. Hepatol.* **2017**, *5*, 223–237. [CrossRef]
136. Malinen, M.M.; Kauttonen, A.; Beaudoin, J.J.; Sjöstedt, N.; Honkakoski, P.; Brouwer, K.L.R. Novel in Vitro Method Reveals Drugs That Inhibit Organic Solute Transporter Alpha/Beta (OSTα/β). *Mol. Pharm.* **2018**, *16*, 238–246. [CrossRef] [PubMed]
137. Kis, E.; Ioja, E.; Nagy, T.; Szente, L.; Herédi-Szabó, K.; Krajcsi, P. Effect of Membrane Cholesterol on BSEP/Bsep Activity: Species Specificity Studies for Substrates and Inhibitors. *Drug Metab. Dispos.* **2009**, *37*, 1878–1886. [CrossRef] [PubMed]
138. Morgan, R.E.; Trauner, M.; Van Staden, C.J.; Lee, P.H.; Ramachandran, B.; Eschenberg, M.; Afshari, C.A.; Qualls, C.W.; Lightfoot-Dunn, R.; Hamadeh, H.K. Interference with Bile Salt Export Pump Function Is a Susceptibility Factor for Human Liver Injury in Drug Development. *Toxicol. Sci.* **2010**, *118*, 485–500. [CrossRef]
139. Marion, T.L.; Perry, C.H.; St Claire, R.L., 3rd; Brouwer, K.L. Endogenous bile acid disposition in rat and human sandwich-cultured hepatocytes. *Toxicol. Appl. Pharmacol.* **2012**, *261*, 1–9. [CrossRef]
140. Hendriks, D.F.; Fredriksson Puigvert, L.; Messner, S.; Mortiz, W.; Ingelman-Sundberg, M. Hepatic 3D spheroid models for the de-tection and study of compounds with cholestatic liability. *Sci. Rep.* **2016**, *6*, 35434. [CrossRef]
141. Ramaiahgari, S.C.; Ferguson, S.S. Organotypic 3D HepaRG Liver Model for Assessment of Drug-Induced Cholestasis. *Methods Mol. Biol.* **2019**, *1981*, 313–323. [CrossRef]
142. Schadt, S.; Simon, S.; Kustermann, S.; Boess, F.; McGinnis, C.; Brink, A.; Lieven, R.; Fowler, S.; Youdim, K.; Ullah, M.; et al. Minimizing DILI risk in drug discovery—A screening tool for drug candidates. *Toxicol. Vitr.* **2015**, *30*, 429–437. [CrossRef] [PubMed]
143. Morgan, R.E.; van Staden, C.J.; Chen, Y.; Kalyanaraman, N.; Kalanzi, J.; Dunn, R.T., 2nd; Afshari, C.A.; Hamadeh, H.K. A multifactorial approach to hepatobiliary transporter assessment enables improved therapeutic compound development. *Toxicol. Sci.* **2013**, *136*, 216–241. [CrossRef] [PubMed]
144. Kenna, J.G.; Taskar, K.S.; Battista, C.; Bourdet, D.L.; Brouwer, K.L.; Brouwer, K.R.; Dai, D.; Funk, C.; Hafey, M.J.; Lai, Y.; et al. Can Bile Salt Export Pump Inhibition Testing in Drug Discovery and Development Reduce Liver Injury Risk? An International Transporter Consortium Perspective. *Clin. Pharmacol. Ther.* **2018**, *104*, 916–932. [CrossRef] [PubMed]
145. Riede, J.; Poller, B.; Huwyler, J.; Camenisch, G. Assessing the Risk of Drug-Induced Cholestasis Using Unbound Intrahepatic Concentrations. *Drug Metab. Dispos.* **2017**, *45*, 523–531. [CrossRef]
146. Yucha, R.W.; He, K.; Shi, Q.; Cai, L.; Nakashita, Y.; Xia, C.Q.; Liao, M. In Vitro Drug-Induced Liver Injury Prediction: Criteria Opti-mization of Efflux Transporter IC50 and Physicochemical Properties. *Toxicol. Sci.* **2017**, *157*, 487–499. [CrossRef]
147. Aleo, M.D.; Shah, F.; Allen, S.; Barton, H.A.; Costales, C.; Lazzaro, S.; Leung, L.; Nilson, A.; Obach, R.S.; Rodrigues, A.D.; et al. Moving beyond Binary Predictions of Human Drug-Induced Liver Injury (DILI) toward Contrasting Relative Risk Potential. *Chem. Res. Toxicol.* **2019**, *33*, 223–238. [CrossRef]

148. Hafey, M.J.; Houle, R.; Tanis, K.Q.; Knemeyer, I.; Shang, J.; Chen, Q.; Baudy, A.; Monroe, J.; Sistare, F.D.; Evers, R. A Two-Tiered In Vitro Approach to De-Risk Drug Candidates for Potential Bile Salt Export Pump Inhibition Liabilities in Drug Discovery. *Drug Metab. Dispos.* **2020**, *48*, 1147–1160. [CrossRef]
149. Garzel, B.; Yang, H.; Zhang, L.; Huang, S.-M.; Polli, J.E.; Wang, H. The Role of Bile Salt Export Pump Gene Repression in Drug-Induced Cholestatic Liver Toxicity. *Drug Metab. Dispos.* **2013**, *42*, 318–322. [CrossRef]
150. Notenboom, S.; Weigand, K.M.; Proost, J.H.; van Lipzig, M.M.; van de Steeg, E.; Broek, P.H.V.D.; Greupink, R.; Russel, F.G.; Groothuis, G.M. Development of a mechanistic biokinetic model for hepatic bile acid handling to predict possible cholestatic effects of drugs. *Eur. J. Pharm. Sci.* **2018**, *115*, 175–184. [CrossRef]
151. Watkins, P.B. The DILI-sim Initiative: Insights into Hepatotoxicity Mechanisms and Biomarker Interpretation. *Clin. Transl. Sci.* **2019**, *12*, 122–129. [CrossRef]
152. Köck, K.; Ferslew, B.C.; Netterberg, I.; Yang, K.; Urban, T.J.; Swaan, P.; Stewart, P.W.; Brouwer, K.L.R. Risk Factors for Development of Cholestatic Drug-Induced Liver Injury: Inhibition of Hepatic Basolateral Bile Acid Transporters Multidrug Resistance-Associated Proteins 3 and 4. *Drug Metab. Dispos.* **2013**, *42*, 665–674. [CrossRef] [PubMed]
153. Chan, R.; Benet, L.Z. Measures of BSEP Inhibition In Vitro Are Not Useful Predictors of DILI. *Toxicol. Sci.* **2017**, *162*, 499–508. [CrossRef] [PubMed]
154. Yoshikado, T.; Takada, T.; Yamamoto, T.; Yamaji, H.; Ito, K.; Santa, T.; Yokota, H.; Yatomi, Y.; Yoshida, H.; Goto, J.; et al. Itraconazole-induced cholestasis: Involvement of the inhibition of bile canalicular phospholipid translocator MDR3/ABCB4. *Mol. Pharmacol.* **2011**, *79*, 241–250. [CrossRef] [PubMed]
155. Mahdi, Z.M.; Synal-Hermanns, U.; Yoker, A.; Locher, K.P.; Stieger, B. Role of Multidrug Resistance Protein 3 in Antifungal-Induced Cholestasis. *Mol. Pharmacol.* **2016**, *90*, 23–34. [CrossRef]
156. Sissung, T.M.; Huang, P.A.; Hauke, R.J.; McCrea, E.M.; Peer, C.J.; Barbier, R.H.; Strope, J.D.; Ley, A.M.; Zhang, M.; Hong, J.A.; et al. Severe Hepatotoxicity of Mith-ramycin Therapy Caused by Altered Expression of Hepatocellular Bile Transporters. *Mol. Pharmacol.* **2019**, *96*, 158–167. [CrossRef]
157. Jackson, J.P.; Brouwer, K.R. The C-DILI™ Assay: An Integrated In Vitro Approach to Predict Cholestatic Hepatotoxicity. In *Experimental Cholestasis Research*; Humana: New York, NY, USA, 2019; pp. 75–85. [CrossRef]
158. Weaver, R.J.; Blomme, E.A.; Chadwick, A.; Copple, I.M.; Gerets, H.H.J.; Goldring, C.E.; Guillouzo, A.; Hewitt, P.G.; Ingelman-Sundberg, M.; Jensen, K.G.; et al. Managing the challenge of drug-induced liver injury: A roadmap for the development and deployment of preclinical predictive models. *Nat. Rev. Drug Discov.* **2019**, *19*, 131–148. [CrossRef]
159. Sticova, E.; Jirsa, M. ABCB4 disease: Many faces of one gene deficiency. *Ann. Hepatol.* **2020**, *19*, 126–133. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.