REVIEW PAPER

Deciphering the On-Target and Off-Target Mechanisms of ADC-Induced Hepatotoxicity: A Systems Pharmacology Approach

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ABSTRACT

Antibody-drug conjugates (ADCs) have rapidly become one of the most transformative classes of oncology therapeutics. With more than fifteen agents now approved by the U.S. Food and Drug Administration (FDA) and hundreds more advancing through clinical pipelines, these molecules have moved well beyond proof-of-concept into mainstream cancer care. Their fundamental appeal lies in a deceptively elegant idea: pair the precision targeting of a monoclonal antibody with the killing power of a cytotoxic payload. In practice, however, the biology is far more complicated than the design principle suggests, and nowhere is this complexity more apparent than in the liver.ADC-induced hepatotoxicity spans a broad clinical spectrum—from transient, asymptomatic aminotransferase elevations that resolve without intervention to devastating hepatic veno-occlusive disease (VOD), also called sinusoidal obstruction syndrome (SOS), which can be fatal. Understanding why and how this happens requires disentangling two overlapping but mechanistically distinct categories of injury. On-target hepatotoxicity arises when the ADC binds its intended antigen on hepatic or biliary cells—an unintended consequence of antigen expression in non-malignant tissue. Off-target toxicity, by contrast, reflects the liver's central role in drug metabolism and the vulnerability of hepatocytes, sinusoidal endothelial cells, and Kupffer cells to premature payload release, bystander diffusion, mitochondrial damage, bile acid transporter inhibition, and Fc-mediated immune activation.This review brings together current mechanistic knowledge through a systems pharmacology lens. We draw on physiologically based pharmacokinetic (PBPK) modeling, network pharmacology, molecular docking, transcriptomic analyses, and advanced in vitro hepatic platforms to build an integrated picture of ADC hepatotoxicity. We systematically examine the hepatic safety profiles of approved ADCs, trace the molecular pathways underpinning both categories of injury, assess emerging biomarkers capable of detecting hepatic stress before it becomes clinically apparent, and evaluate strategies for mitigation—including linker-payload engineering, dosing optimization, hepatoprotective agents, and next-generation ADC design approaches such as masked or activatable conjugates and site-specific conjugation. The role of quantitative systems pharmacology (QSP) frameworks in translating mechanistic insight into predictive risk tools is considered throughout. We conclude with regulatory perspectives and a forward-looking research agenda aimed at widening the therapeutic index of ADCs through principled hepatotoxicity management.

Keywords: *antibody-drug conjugate; hepatotoxicity; systems pharmacology; PBPK modeling; veno-occlusive disease; payload toxicity; linker stability; drug-induced liver injury; DILI; translational pharmacology*

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INTRODUCTION

The idea of a therapeutic agent capable of striking malignant cells while leaving healthy tissue untouched has captivated oncologists for well over a century. Antibody-drug conjugates represent the most clinically mature realization of this vision to date. By attaching a potent cytotoxic molecule to a tumor-targeting antibody via a chemical linker, ADC designers have created agents that, at their best, function as guided missiles—delivering lethal payloads directly to cancer cells (Chari, Miller and Widdison, 2014). As of 2025, more than fifteen ADCs have received FDA approval, spanning tumor types from HER2-positive breast cancer and hematologic malignancies to urothelial carcinoma and cervical cancer, with a clinical pipeline exceeding 200 investigational candidates (Drago, Modi and Chandarlapaty, 2021).An ADC is, at its core, a three-part molecule: a monoclonal antibody that confers selectivity, a cytotoxic payload that does the killing, and a linker that holds the two together. The choice of each component is not merely an engineering detail—it shapes the safety profile in ways that are only partially understood. Among the most clinically significant of these consequences is hepatotoxicity (Masters et al., 2018). The liver sits at the intersection of ADC pharmacokinetics and pharmacodynamics in ways that make it uniquely vulnerable. As the primary organ of protein catabolism, drug metabolism, and biliary excretion, it is exposed to high concentrations of the intact ADC, partially metabolized linker-payload fragments, and free cytotoxic molecules that escape the conjugate prematurely. Hepatic sinusoidal endothelial cells, Kupffer cells, hepatocytes, and cholangiocytes each interact with ADC components in distinct ways, and each can be injured by them.The clinical history of gemtuzumab ozogamicin is a sobering illustration of what hepatotoxicity can mean in practice. Originally approved in 2000 for acute myeloid leukemia, gemtuzumab was voluntarily withdrawn from the U.S. market in 2010 after post-marketing studies revealed an unacceptably high rate of fatal hepatic VOD. It returned in 2017 in a reformulated, lower-dose fractionated regimen with a markedly improved safety profile—a testament to what mechanistic understanding, combined with careful pharmaceutical chemistry, can achieve (Bross et al., 2001; Rajvanshi et al., 2002). Similar considerations have shaped the clinical development of inotuzumab ozogamicin and continue to influence the design of emerging ADC platforms.Despite its clinical importance, our mechanistic understanding of ADC-induced hepatotoxicity remains incomplete. The distinction between on-target effects—where the antibody binds its intended antigen on hepatic cells—and off-target effects—arising from non-specific uptake, linker cleavage, payload diffusion, or immune-mediated injury—is conceptually clear but difficult to disentangle in clinical practice, where multiple mechanisms often operate simultaneously. A systems pharmacology approach, integrating computational modeling with multi-omics data and advanced hepatic experimental models, offers a coherent framework for making sense of this complexity, developing biomarkers that can detect injury early, and engineering ADCs with better hepatic safety profiles.This review examines the mechanistic landscape of ADC-induced hepatotoxicity with that goal in mind. We begin with the structural and pharmacological features of ADCs that shape hepatic exposure, move through the on-target and off-target mechanisms of injury, discuss the computational and experimental tools available for mechanistic analysis, evaluate emerging biomarker strategies, and close with translational mitigation frameworks and regulatory considerations

1.0 STRUCTURAL AND PHARMACOLOGICAL DETERMINANTS OF ADC HEPATOTOXICITY

1.1 Antibody Component: FcRn Recycling and Hepatic Accumulation

The immunoglobulin G backbone that virtually all approved ADCs share carries with it an intrinsic predisposition to hepatic accumulation. The neonatal Fc receptor (FcRn), expressed on hepatocytes, liver sinusoidal endothelial cells (LSECs), and Kupffer cells, mediates pH-dependent transcytosis and recycling of IgG molecules—a process that extends antibody half-life but also concentrates ADC at hepatic sites. The practical consequence is that hepatic ADC concentrations can substantially exceed plasma levels, particularly when drug-to-antibody ratios (DARs) are high or half-lives are long (Shen et al., 2012). This FcRn- and Fcγ receptor-mediated hepatic trapping creates conditions in which the liver is simultaneously exposed to the full molecular entity—antibody, linker, and payload—establishing the preconditions for both on-target and off-target injury.The IgG subclass choice matters here as well. IgG1-based ADCs, which predominate in the approved landscape, carry robust Fc effector function and strong Fcγ receptor binding affinity, predisposing to Kupffer cell activation and inflammatory hepatic injury (Perez et al., 2014). Fc-silent or IgG4-based formats can attenuate this hepatic inflammatory signaling, though often at the cost of antibody-dependent cellular cytotoxicity relevant to antitumor efficacy.

1.2 Linker Chemistry and Stability

If the antibody determines where an ADC goes, the linker largely determines what happens once it gets there—at least from a hepatotoxicity standpoint. Linkers fall into two broad categories: cleavable, which release payload in response to specific intracellular stimuli, and non-cleavable, which require complete lysosomal catabolism of the antibody before payload is liberated (Beck et al., 2017). Each category carries distinct hepatic stability implications.Acid-labile hydrazone linkers, as used in gemtuzumab and inotuzumab ozogamicin, were designed to exploit the acidic pH of tumor endolysosomes for payload release. The problem is that hepatic lysosomes and biliary canaliculi are also mildly acidic, providing a physiologically permissive environment for non-specific linker hydrolysis and intrahepatic calicheamicin release (Rajvanshi et al., 2002; Dijoseph et al., 2004). Disulfide linkers, used in maytansinoid-bearing ADCs, rely on the glutathione gradient between plasma and cytoplasm for

stability; the high intracellular GSH content of hepatocytes (~5–10 mM versus plasma's ~5–20 µM) means that disulfide bonds can be reduced during transcellular passage through the liver (Hamblett et al., 2004).Valine-citrulline peptide linkers, used in MMAE-bearing ADCs like brentuximab vedotin and enfortumab vedotin, require cathepsin B-mediated cleavage for payload release. The robust cathepsin B activity present in hepatocytes provides a mechanistic basis for MMAE liberation within the liver, enabling bystander killing of adjacent cells—therapeutically useful in heterogeneous tumors, but hazardous when those adjacent cells are normal hepatocytes (Caculitan et al., 2017). Non-cleavable thioether linkers, as in T-DM1, theoretically confine payload release to the lysosome following complete antibody catabolism, limiting systemic free-drug exposure. Yet T-DM1 causes meaningful transaminase elevations in a substantial minority of patients, pointing to mechanisms that persist even when linker cleavage is not the primary route of payload liberation.

1.3 Payload Properties and Hepatic Metabolism

The cytotoxic payload is the primary driver of ADC hepatotoxic potency. Major payload classes and their hepatic metabolic pathways are as follows. Maytansinoids (DM1, DM4) are metabolized by hepatic CYP3A4 and CYP3A5, producing reactive intermediates that can form adducts with hepatic proteins (Stepan et al., 2011). DM1's disruption of microtubule dynamics impairs hepatocyte morphology, bile canalicular function, and mitochondrial trafficking. Auristatins (MMAE, MMAF) undergo extensive hepatic first-pass metabolism via CYP3A4, and MMAE in particular exhibits significant membrane permeability, enabling bystander diffusion from targeted cells to adjacent hepatocytes (Li et al., 2016). The bystander effect is a double-edged sword: therapeutically beneficial in tumors with heterogeneous antigen expression, but hepatotoxic when the bystander cells are non-malignant hepatocytes.Calicheamicins (as in gemtuzumab and inotuzumab) are among the most potent cytotoxins known, with sub-nanomolar DNA double-strand break activity. Their hepatic toxicity is amplified by the susceptibility of sinusoidal endothelial cells, which lack the DNA repair mechanisms of hepatocytes, and by their tendency to concentrate in the liver through FcRn and CD33/CD22 expression on Kupffer cells (Bross et al., 2001). Camptothecin derivatives such as SN-38 (in sacituzumab govitecan via hydrolyzable CL2A linker) and DXd (in T-DXd) are topoisomerase I inhibitors that can inhibit biliary SN-38 glucuronidation, causing intrahepatic SN-38 accumulation and cholestatic injury (Goldenberg and Sharkey, 2020).

1.4 Overview of Approved ADCs and Their Hepatotoxicity Profiles

Table 1 summarizes the major FDA-approved ADCs, their structural components, and associated hepatotoxicity profiles as reported in pivotal clinical trials and post-marketing surveillance.

Table 1. FDA-Approved ADCs: Structural Components and Hepatotoxicity Profiles

ADC Name	Target	Payload	Linker Type	Indication	Hepatotoxicity Grade
Ado-trastuzumab emtansine (T-DM1)	HER2	DM1 (maytansinoid)	Thioether (SMCC)	HER2+ breast cancer	Grade 1-3 (AST/ALT elevation ~5-8%)
Trastuzumab deruxtecan (T-DXd)	HER2	DXd (topoisomerase I inhibitor)	Cleavable tetrapeptide	HER2+ breast/gastric	Grade 1-2 (transaminase ~3-5%)
Brentuximab vedotin	CD30	MMAE (auristatin)	Cleavable val-cit	HL, ALCL	Grade 1-3 liver injury reported
Enfortumab vedotin	Nectin-4	MMAE	Cleavable val-cit	Urothelial carcinoma	Rare Grade 3-4 ALT elevation
Sacituzumab govitecan	TROP-2	SN-38 (irinotecan metabolite)	Hydrolyzable CL2A	TNBC, urothelial	Grade 1-2 bilirubin elevation
Polatuzumab vedotin	CD79b	MMAE	Cleavable val-cit	DLBCL	Transaminase elevation Grade 1-2
Loncastuximab tesirine	CD19	PBD dimer	Cleavable val-ala	DLBCL	Rare hepatotoxicity

Belantamab mafodotin	BCMA	MMAF (auristatin F)	Non-cleavable MC	RRMM	Hepatotoxicity rare; ocular dominant
Gemtuzumab ozogamicin	CD33	Calicheamicin	Hydrazone (acid-labile)	AML	VOD/SOS in up to 15-20%
Inotuzumab ozogamicin	CD22	Calicheamicin	Hydrazone	B-ALL	VOD/SOS up to 15%; Grade 3-4 common
Mirvetuximab soravtansine	FRα	DM4 (maytansinoid)	Disulfide SPDB	Ovarian cancer	Grade 1-2 AST/ALT elevation
Disitamab vedotin	HER2	MMAE	Cleavable	Gastric/GEJ cancer	Transaminase elevation reported

Table 1. Summary of FDA-approved ADCs with structural characteristics and associated hepatotoxicity findings from pivotal trials. AST = aspartate aminotransferase; ALT = alanine aminotransferase; HL = Hodgkin lymphoma; ALCL = anaplastic large cell lymphoma; DLBCL = diffuse large B-cell lymphoma; TNBC = triple-negative breast cancer; RRMM = relapsed/refractory multiple myeloma; AML = acute myeloid leukemia; VOD = veno-occlusive disease; SOS = sinusoidal obstruction syndrome.

2. ON-TARGET MECHANISMS OF ADC-INDUCED HEPATOTOXICITY

2.1 Antigen Expression on Hepatobiliary Cells

On-target hepatotoxicity arises when the antibody component of an ADC binds to its intended antigen on non-malignant hepatic cells, triggering receptor-mediated internalization and intracellular payload delivery. This mechanism is particularly relevant when the target antigen is expressed on hepatocytes, cholangiocytes, or sinusoidal cells at physiologically significant levels. HER2, the target of T-DM1 and trastuzumab deruxtecan, is expressed at low-to-moderate levels on bile duct epithelial cells and hepatocytes, providing a mechanistic basis for the transaminase elevations observed with these agents (Verma et al., 2012; Modi et al., 2020).CD33 expression on hepatic Kupffer cells and sinusoidal macrophages explains the predilection of gemtuzumab ozogamicin for hepatic sinusoidal injury. When gemtuzumab binds CD33 on these cells, internalization delivers calicheamicin directly to endolysosomal compartments, where linker cleavage occurs at low pH. The released calicheamicin then damages DNA in both the targeted cells and, through diffusion, in adjacent hepatocytes and sinusoidal endothelial cells (Rajvanshi et al., 2002). CD22, targeted by inotuzumab ozogamicin, similarly shows hepatic expression patterns that contribute to on-target VOD through sinusoidal macrophage involvement (Norsworthy et al., 2019).

2.2 FcRn-Mediated Hepatic Uptake as an On-Target Process

The FcRn receptor, expressed on hepatocytes and LSECs, mediates not only IgG recycling but also endocytosis of intact ADC molecules, constituting an on-target hepatic accumulation mechanism independent of the antigen-binding specificity. This is because FcRn binds the Fc region—common to all IgG-based ADCs—in a pH-dependent manner, resulting in acidic endosomal trapping followed by lysosomal delivery of the intact ADC. Within the lysosome, linker cleavage (for cleavable ADCs) or complete antibody catabolism (for non-cleavable ADCs) releases the payload, which can then interact with hepatic organelles and gene expression machinery (Shen et al., 2012; Deng et al., 2016).PBPK modeling incorporating FcRn dynamics has demonstrated that liver-to-plasma concentration ratios for ADC payloads can exceed 10-fold in preclinical models, underscoring the importance of FcRn-mediated hepatic accumulation as a pharmacokinetic driver of hepatotoxicity risk (Zhao et al., 2020). This finding has important implications for ADC design, as modifications that reduce FcRnaffinity—such as the YTE mutation in the Fc region—can alter the hepatic accumulation profile and potentially reduce hepatotoxicity.

2.3 Cholangiocyte Injury and Cholestasis

Cholangiocytes lining the intrahepatic bile ducts express several antigens targeted by approved ADCs, including HER2 and TROP-2. On-target binding to cholangiocytes with subsequent intracellular payload release can disrupt bile acid transport, tight junction integrity, and bile duct morphology. SN-38, the active metabolite released from sacituzumab govitecan, is a known inhibitor of the bile salt export pump

(BSEP/ABCB11) and the multidrug resistance protein 2 (MRP2/ABCC2), both critical for biliary excretion of conjugated bile acids and drug metabolites (Goldenberg and Sharkey, 2020). Inhibition of these transporters produces an intrahepatic cholestatic pattern characterized by elevated alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and direct bilirubin, which can progress to biliary fibrosis with prolonged exposure.

3. OFF-TARGET MECHANISMS OF ADC-INDUCED HEPATOTOXICITY

3.1 Payload Bystander Toxicity

Bystander toxicity represents one of the most mechanistically significant off-target hepatotoxic pathways. Following receptor-mediated internalization into tumor or non-tumor cells expressing the target antigen, cleavable linkers such as val-cit are hydrolyzed by lysosomal cathepsins, releasing membrane-permeable payload molecules (e.g., MMAE). These small-molecule payloads can then diffuse across cell membranes into adjacent non-targeted hepatocytes, delivering cytotoxic effects to cells that may not express the target antigen. In vitro studies using HepaRG cells and primary human hepatocyte co-culture models have demonstrated that MMAE-releasing ADCs cause measurable hepatocyte cytotoxicity at clinically relevant concentrations, even in the absence of target antigen expression on hepatocytes (Li et al., 2016; Kolhatkar et al., 2021).The bystander effect magnitude depends on payload membrane permeability, cellular efflux capacity (P-glycoprotein/ABCB1 expression), and the spatial density of antigen-expressing cells within the hepatic microenvironment. MMAF, the charged auristatin derivative used in belantamab mafodotin, exhibits significantly reduced membrane permeability compared to MMAE due to its positively charged phenylalanine residue, resulting in a substantially attenuated bystander effect and reduced hepatotoxicity—illustrating how payload molecular design directly influences off-target hepatic safety (Hamblett et al., 2015).

3.2 Linker Instability and Free Payload Release

The systemic stability of the ADC linker is a critical determinant of off-target hepatotoxicity. Premature linker cleavage in plasma releases free cytotoxic payload that distributes according to its intrinsic pharmacokinetic properties rather than the antibody's antigen-guided targeting. Free payload molecules exhibit substantially different distribution volumes, metabolic pathways, and hepatic clearance characteristics than their conjugated counterparts, and this pharmacokinetic decoupling creates a scenario in which the liver—as the primary organ of drug metabolism—is exposed to disproportionate free-drug concentrations (Hamblett et al., 2004).The original formulation of gemtuzumab ozogamicin (Mylotarg) used a fast-loading synthesis process resulting in substantial free calicheamicin content and linker heterogeneity. Post-marketing analyses demonstrated that the resulting systemic calicheamicin exposure contributed significantly to VOD risk,particularly in patients receiving the agent as a bridge to hematopoietic stem cell transplantation. The reformulated, lower-dose fractionated regimen approved in 2017 substantially reduced free-drug content and the incidence of severe VOD through more controlled linker-payload loading (Bross et al., 2001).

3.3 Mitochondrial Dysfunction

Microtubule-targeting payloads, including maytansinoids (DM1, DM4) and auristatins (MMAE, MMAF), induce mitochondrial dysfunction in hepatocytes through mechanisms distinct from their intended antimitotic activity. DM1 disrupts the mitochondria-associated cytoskeletal network, impairing mitochondrial fission/fusion dynamics and reducing electron transport chain efficiency. In HepaRG cell models treated with T-DM1 catabolites, Seahorse extracellular flux analysis has demonstrated significant reductions in basal and maximal oxygen consumption rates, accompanied by elevated reactive oxygen species (ROS) production and mitochondrial membrane potential collapse (Kolhatkar et al., 2021). These findings are consistent with the pattern of hepatocellular ATP depletion and oxidative stress observed in T-DM1-associated hepatotoxicity.Camptothecin-derived payloads such as DXd (in T-DXd) and SN-38 (in sacituzumab govitecan) also exert mitochondrial toxicity through topoisomerase I inhibition in mitochondrial DNA, which is particularly rich in replication intermediates susceptible to topoisomerase I-mediated DNA strand breaks. This mitochondrially mediated cytotoxicity contributes to the transaminase elevations observed with these agents, particularly at higher dose levels (Ogitani et al., 2016).

3.4 Bile Acid Transporter Inhibition

A mechanistically distinct off-target pathway involves inhibition of hepatocanalicular bile acid transporters by ADC payloads or their metabolites. BSEP (ABCB11) is the primary hepatocanalicular transporter responsible for bile salt excretion, and its inhibition causes intrahepatic accumulation of hydrophobic bile acids, leading to cholangiocyte injury, hepatocyte apoptosis, and progressive cholestasis. In vitro membrane vesicle

transport assays have demonstrated inhibitory activity against BSEP for DM1 metabolites (IC50 ~5–15 μM), SN-38 (IC50 ~10–30 μM), and free MMAE (IC50 ~20–50 μM), with clinically relevant concentrations in the hepatic portal circulation potentially approaching these thresholds during peak drug exposure (Li et al., 2021).The sodium-taurocholate cotransporting polypeptide (NTCP/SLC10A1), responsible for hepatocellular uptake of conjugated bile acids from portal blood, represents another potential target of ADC payload inhibition. Inhibition of NTCP by payload metabolites could contribute to systemic bile acid retention and hypercholanemia, exacerbating systemic symptoms of cholestasis. MRP2 (ABCC2), which mediates biliary excretion of glucuronide conjugates including bilirubin glucuronide, is inhibited by several camptothecin metabolites, contributing to the hyperbilirubinemia observed with sacituzumab govitecan (Goldenberg and Sharkey, 2020).

3.5 Immune-Mediated Hepatic Injury

The Fc domain of ADC antibodies interacts with Fcγ receptors (FcγRI, FcγRIII) expressed on hepatic Kupffer cells—the liver's resident macrophage population comprising approximately 15–20% of non-parenchymal liver cells. Fc-FcγR ligation activates Kupffer cells, triggering release of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6, and reactive oxygen intermediates, which can damage adjacent hepatocytes and sinusoidal endothelial cells through a paracrine inflammatory mechanism. This immune-mediated pathway is particularly relevant for calicheamicin-bearing ADCs, where Kupffer cell activation may be compounded by direct calicheamicin-mediated DNA damage in these cells following internalization (Rajvanshi et al., 2002).The complement system may also be activated by ADC-immune complexes deposited in hepatic sinusoids, producing a membrane attack complex that contributes to sinusoidal endothelial injury. C3a and C5a anaphylatoxins generated by complement activation further amplify the local inflammatory response, contributing to the hepatic inflammatory infiltrate observed in ADC-related liver biopsies. This immune-inflammatory amplification loop is particularly relevant in patients with pre-existing hepatic inflammation (chronic hepatitis, fatty liver disease), who may exhibit exaggerated responses to ADC-mediated immune activation.

3.6 Veno-Occlusive Disease / Sinusoidal Obstruction Syndrome

Hepatic veno-occlusive disease (VOD), now more precisely termed sinusoidal obstruction syndrome (SOS) to reflect its primary pathoanatomic site, represents the most severe form of ADC-induced hepatotoxicity and is associated with significant mortality, particularly when superimposed on conditioning regimens for hematopoietic stem cell transplantation (HSCT) (Kharfan-Dabaja et al., 2013). The pathogenesis of ADC-induced VOD involves selective injury to centrilobular hepatic sinusoidal endothelial cells (LSECs), which lack the regenerative capacity of hepatocytes and are particularly vulnerable to cytotoxic injury.LSEC injury disrupts the sinusoidal fenestration architecture, enabling subendothelial edema, coagulation activation, and deposition of red cell debris in the space of Disse. Progressive sinusoidal narrowing and eventual obliteration of terminal hepatic venules produces hepatic congestion, ascites, hyperbilirubinemia, and weight gain—the clinical hallmarks of VOD. In severe cases, multi-organ failure ensues. Gemtuzumab ozogamicin and inotuzumab ozogamicin carry FDA black box warnings for VOD, with incidences ranging from 10–15% in recent trials (Norsworthy et al., 2019), substantially higher in post-HSCT settings.

3.7 Mechanistic Summary

Table 2 provides a comprehensive mechanistic classification of ADC-induced hepatotoxicity, encompassing both on-target and off-target pathways with supporting evidence and implicated agents.

Table 2. Mechanistic Classification of ADC-Induced Hepatotoxicity

Mechanism Category	Key Molecular Event	ADC(s) Implicated	Clinical/Preclinical Evidence
On-target (target-mediated)	FcRn-mediated endocytosis in hepatocytes; HER2/CD33 expression on hepatic cells	T-DM1, Gemtuzumab	Liver biopsies showing sinusoidal injury; target expression in biliary cells

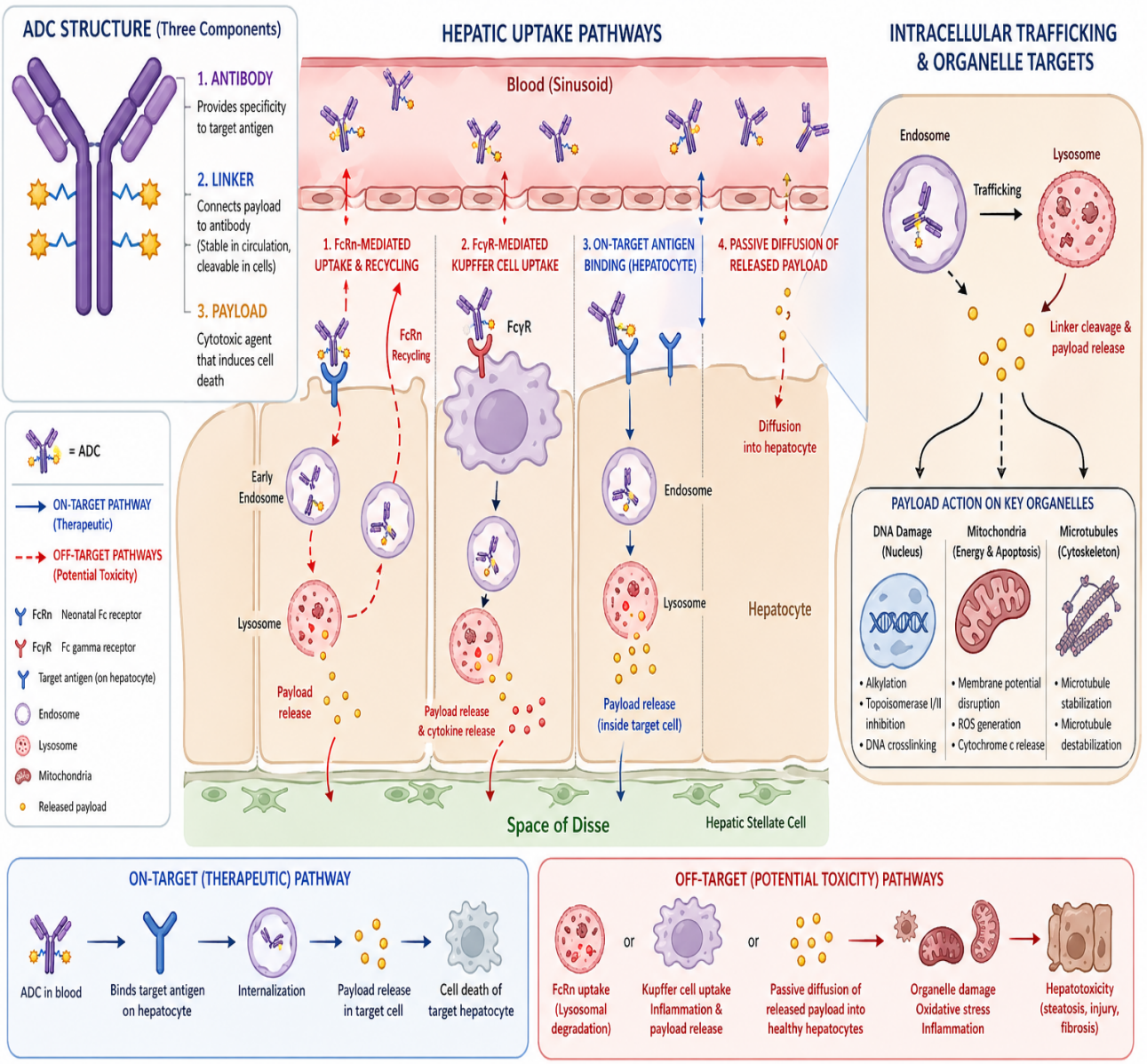
Payload bystander toxicity	Diffusion of cytotoxic payload (MMAE, DM1) to adjacent hepatocytes after linker cleavage	Brentuximab, T-DM1	In vitro hepatocyte models; elevated ALT in xenograft studies
Linker instability / premature cleavage	Acid-labile or reducible linkers cleave in bloodstream; free payload reaches liver	Gemtuzumab (hydrazone)	PK studies showing free calicheamicin; high VOD incidence with early formulations
Mitochondrial dysfunction	DM1/DM4 interfere with tubulin dynamics in hepatocytes; MMAE disrupts cellular energy	T-DM1, T-DXd	Seahorse assay data; ROS elevation in HepaRG cells
Bile acid transporter inhibition	Payload metabolites inhibit BSEP, MRP2, NTCP affecting bile flow	SN-38 (sacituzumab)	BSEP IC50 data; cholestatic pattern on LFTs
Immune-mediated / Fc-receptor	ADCC via hepatic Kupffer cell FcγR activation; cytokine release	Calicheamicin ADCs	Kupffer cell activation markers; TNF-α/IL-6 elevation
Veno-occlusive disease (VOD/SOS)	Sinusoidal endothelial injury, hepatic venule obstruction	Gemtuzumab, Inotuzumab	Histopathology; defibrotide treatment responses
Oxidative stress / GSH depletion	Reactive intermediates from payload metabolism deplete glutathione	DM1, calicheamicin	Nrf2/HO-1 pathway upregulation in HepG2 cells
Drug-drug interaction (CYP450)	DM1/MMAE metabolism via CYP3A4; interactions with concomitant hepatotoxins	T-DM1 + taxanes	Population PK modeling; CYP3A4 inhibitor studies
Epigenetic dysregulation	PBD dimers cause DNA crosslinks in hepatocytes; chromatin remodeling	Loncastuximab	Comet assay; γH2AX foci in human hepatocytes

Table 2. Comprehensive mechanistic classification of on-target and off-target pathways of ADC-induced hepatotoxicity. BSEP = bile salt export pump; MRP2 = multidrug resistance-associated protein 2; NTCP = sodium-taurocholate cotransporting polypeptide; ROS = reactive oxygen species; VOD = veno-occlusive disease; SOS = sinusoidal obstruction syndrome; GSH = glutathione; PBD = pyrrolobenzodiazepine.

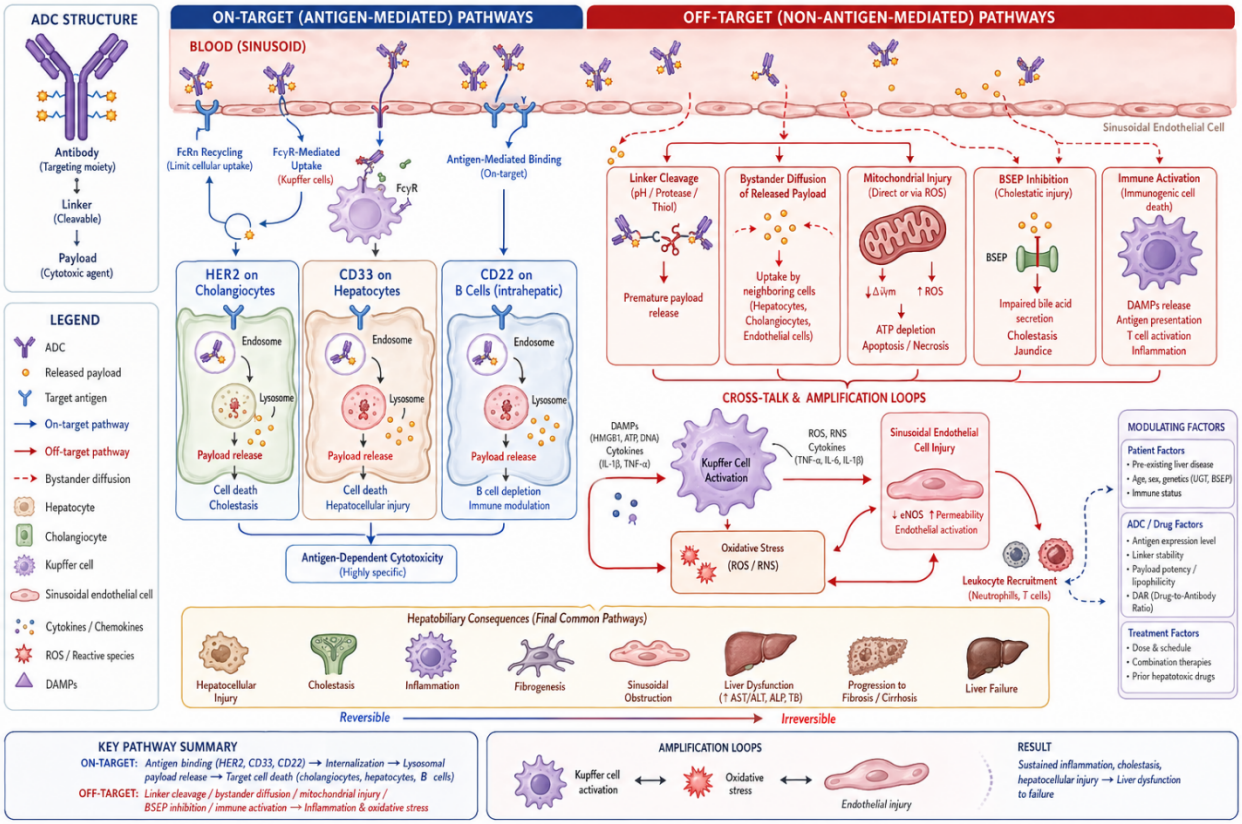
4. SCHEMATIC REPRESENTATIONS OF KEY MECHANISTIC PATHWAYS

The following figures illustrate the principal mechanistic pathways of ADC-induced hepatotoxicity, the systems pharmacology framework for mechanistic analysis, and the integration of multi-scale data for predictive toxicity assessment.

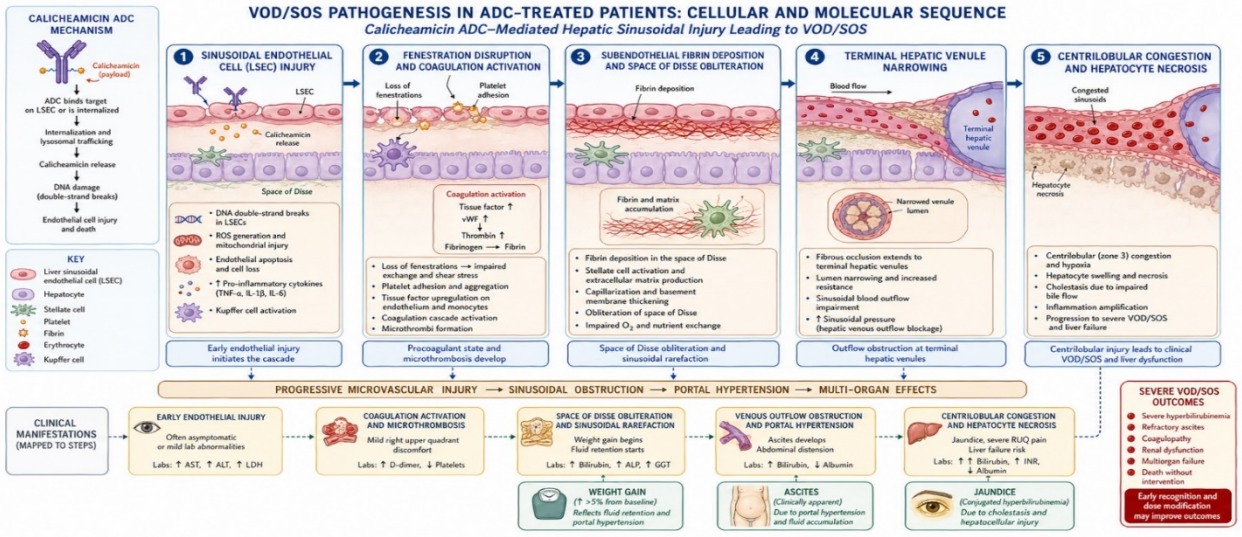
FIGURE 1: Structural Architecture of an ADC and Hepatic Pharmacokinetic Fate



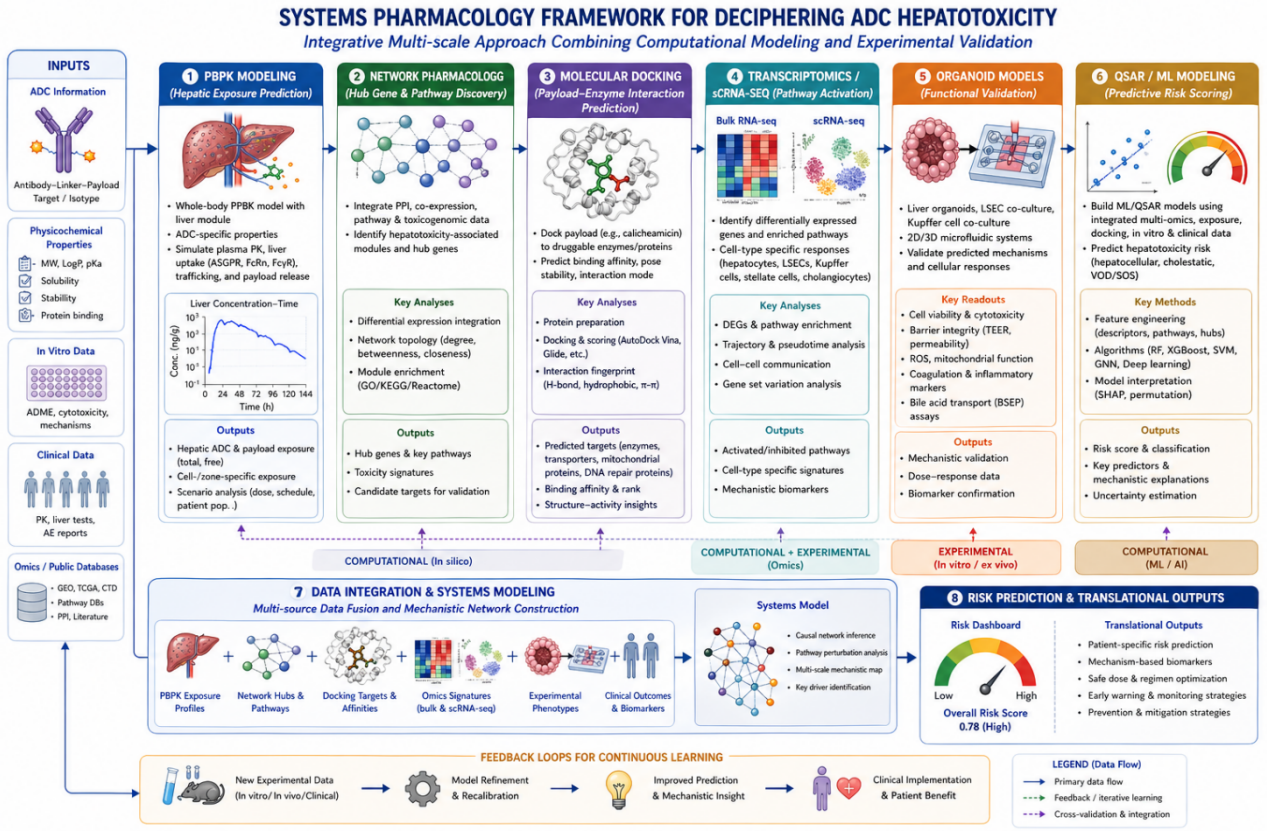
[FIGURE 2: On-Target vs Off-Target Hepatotoxicity Pathways: Integrated Mechanistic Map]



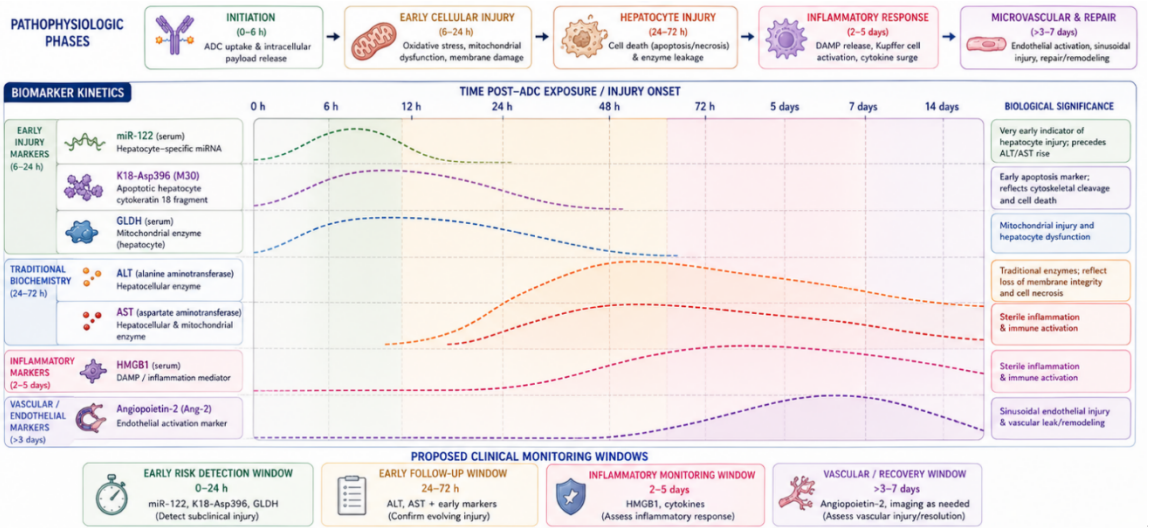
[FIGURE 3: VOD/SOS Pathogenesis in ADC-Treated Patients: Cellular and Molecular Sequence]



[FIGURE 4: Systems Pharmacology Framework for Deciphering ADC Hepatotoxicity]



[FIGURE 5: Emerging Biomarker Panel for ADC Hepatotoxicity: Timeline of Elevation Relative to Injury]



4.0 SYSTEMS PHARMACOLOGY APPROACHES TO MECHANISTIC DISSECTION

4.1 Physiologically Based Pharmacokinetic (PBPK) Modeling

PBPK modeling has emerged as a cornerstone of quantitative systems pharmacology (QSP) applied to ADC hepatotoxicity. By integrating physiological parameters (hepatic blood flow, biliary clearance, FcRn expression density, lysosomal pH, cathepsin B activity), drug-specific parameters (payload lipophilicity, protein binding, CYP metabolism), and ADC-specific parameters (DAR distribution, linker cleavage kinetics, antibody-antigen binding affinity), PBPK models can predict the hepatic concentration-

time profiles of both intact ADC and released payload with substantially greater mechanistic fidelity than traditional compartmental models (Zhao et al., 2020; Guo et al., 2019).Validated PBPK models for T-DM1 have demonstrated that hepatic DM1 (free payload) concentrations can reach 20–50-fold higher than plasma free DM1 concentrations, driven by the combination of FcRn-mediated hepatic uptake of the intact ADC and subsequent lysosomal catabolism. These models predict that patients with hepatic impairment (Child-Pugh B/C) exhibit significantly elevated hepatic payload exposure due to reduced lysosomal catabolism and impaired biliary excretion of DM1 metabolites—findings with direct clinical implications for dose reduction guidelines in this population (Singh et al., 2020).More sophisticated multi-scale PBPK models have incorporated intracellular trafficking kinetics (endosomal sorting, lysosomal residence time, nuclear vs cytoplasmic payload fate), enabling prediction of not only peak hepatic concentrations but also the sub-cellular distribution of payload molecules across distinct hepatic cell populations (hepatocytes, LSECs, Kupffer cells, cholangiocytes). These spatially resolved models represent a significant advance for identifying the cell population most vulnerable to payload-mediated injury for a given ADC design (Guo et al., 2019).

4.2 Network Pharmacology

Network pharmacology constructs interaction graphs integrating ADC components, hepatic target genes, metabolic enzymes, and signaling pathways to identify central hub nodes that mediate hepatotoxicity mechanisms. When applied to ADC hepatotoxicity, network analyses consistently identify several hub genes and pathways: TP53 (DNA damage response integration), CYP3A4/CYP3A5 (payload metabolism), BSEP/ABCB11 (cholestatic injury), NF-κB (inflammatory amplification),Nrf2/KEAP1 (oxidative stress response), BCL-2 family (apoptosis/necrosis decision), and the mitogen-activated protein kinase (MAPK) cascade (Weiler et al., 2021).Network-based analyses of transcriptomic data from ADC-treated hepatocyte models have revealed that the primary differentially expressed pathways include oxidative phosphorylation (downregulated), bile acid metabolism (disrupted), and NF-κB signaling (upregulated), with strong pathway-level convergence across structurally distinct ADC payloads. This pathway-level convergence suggests common mechanistic denominators that could serve as targets for broad-spectrum hepatoprotective interventions, irrespective of the specific payload being used (Weiler et al., 2021).

4.3 Molecular Docking Studies

Molecular docking analyses have provided atomic-level insights into ADC payload interactions with hepatically expressed enzymes and transporters. CYP3A4, the predominant hepatic metabolizer of both MMAE and DM1, has been extensively docked with these payloads, revealing high-affinity binding interactions with the active site heme iron and key substrate recognition residues (SRS1-6). Docking simulations predict IC50 values for competitive CYP3A4 inhibition by DM1 in the low micromolar range, suggesting that supratherapeutic hepatic DM1 concentrations could produce mechanism-based inhibition of CYP3A4, with implications for drug-drug interactions with co-administered hepatotoxins metabolized by the same enzyme (Li et al., 2021).Docking studies of SN-38 (sacituzumab govitecan payload) with BSEP have identified a binding mode within the nucleotide-binding domain 2 that competitively inhibits bile salt translocation, providing structural validation for the cholestatic hepatotoxicity profile of sacituzumab govitecan. Similarly, calicheamicin γ1I has been modeled in silico with hepatic DNA repair enzymes, revealing preferential binding to the minor groove of AT-rich sequences in mitochondrial DNA—an interaction that may explain the selective sinusoidal endothelial cell vulnerability of calicheamicin, as these cells exhibit reduced mitochondrial DNA repair capacity compared to hepatocytes (Dijoseph et al., 2004; Li et al., 2021).

4.4 Transcriptomics and Single-Cell Approaches

Transcriptomic profiling of human hepatocyte models, liver organoids, and clinical liver biopsy specimens from ADC-treated patients has provided mechanistic insights into hepatotoxicity pathways atthe systems level. RNA sequencing of HepaRG cells treated with T-DM1 catabolites identified significant upregulation of the oxidative stress response (NRF2 target genes: HMOX1, NQO1, GCLM), mitochondrial unfolded protein response (ATF5, CHOP, HSP60), and DNA damage checkpoint genes (CDKN1A/p21, GADD45A), while bile acid synthesis genes (CYP7A1, CYP8B1) were significantly downregulated, consistent with the clinical pattern of transaminase elevation without significant cholestasis for T-DM1 (Kolhatkar et al., 2021).Single-cell RNA sequencing (scRNA-seq) of human liver organoids and primary liver samples treated with ADC catabolites has enabled unprecedented resolution of cell type-specific vulnerability patterns. These analyses demonstrate that centrilobular hepatocytes (zone 3, high CYP3A4 expression) are preferentially injured by maytansinoid payloads due to higher intracellular payload accumulation driven by CYP3A4-mediated reactive intermediate formation. In contrast, sinusoidal endothelial cell clusters show selective vulnerability to calicheamicin, consistent with the VOD pathology, while cholangiocytes show greatest susceptibility to SN-38 and BSEP inhibitors (Sevigny et al., 2022).

4.5 Advanced Hepatic In Vitro Models

The accurate recapitulation of ADC hepatotoxicity in vitro requires hepatic models that capture the complexity of the hepatic microenvironment, including cell-cell interactions, bile canalicular architecture, and immune components. Three-dimensional liver organoids derived from human primary hepatocytes or induced pluripotent stem cells (iPSCs) maintain hepatocyte polarity, bile canalicular networks, and cytochrome P450 activity for extended culture periods, enabling long-term exposure studies that better reflect clinical ADC dosing intervals (Sevigny et al., 2022).Liver-on-a-chip microphysiological systems (MPS) incorporating hepatocytes, LSECs, Kupffer cells, and stellate cells in a perfused microfluidic format have demonstrated the capacity to recapitulate ADC-induced sinusoidal injury, cholestasis, and inflammatory activation in a single platform. These systems enable real-time monitoring of bile acid secretion, albumin production, cytochrome P450 activity, and cytokine release simultaneously, providing multi-endpoint toxicity readouts that more closely mimic the clinical hepatotoxicity spectrum than simple 2D hepatocyte cytotoxicity assays (Kolhatkar et al., 2021).

Table 3. Systems Pharmacology Tools Applied to ADC Hepatotoxicity Research

Computational Tool / Method	Application in ADC Hepatotoxicity	Key Outputs	Limitations
PBPK Modeling (GastroPlus, Simcyp)	Predicts hepatic exposure of payload, linker metabolites; FcRn recycling	AUC liver, predicted DILI risk scores	Limited ADC-specific parameters; FcRn dynamics not fully captured
Network Pharmacology (STRING, Cytoscape)	Maps payload-target-liver gene interaction networks; identifies hub genes	Hub gene networks (TP53, CYP3A4, BSEP)	Functional validation required; static networks
Molecular Docking (AutoDock, Glide)	Predicts payload binding to hepatic enzymes (CYP3A4, BSEP, NTCP)	Binding affinities, interaction maps	Flexibility limitations; solvation approximations
Transcriptomics / RNA-seq	Identifies differentially expressed hepatotoxicity pathways	DEG lists, pathway enrichment (KEGG, GO)	Batch effects; transcriptome ≠ proteome
DILI Predictive Models (DILIsym, QSAR)	QSAR models predict payload hepatotoxicity from structure	Hepatotoxicity probability scores	Training data bias; scaffold limitations
Organoid / Microphysiological Systems	3D liver organoids recapitulate ADC-induced sinusoidal/cholangiocyte injury	IC50 values, viability profiles	Scalability; lacks immune components
Machine Learning / Deep Learning	Integrates multi-omics + PK data to predict hepatotoxic ADC features	Predictive classifiers, feature importance	Black-box; interpretability challenges
Single-cell RNA sequencing (scRNA-seq)	Resolves hepatocyte subpopulation-specific vulnerability to payload	Cell cluster toxicity maps	Expensive; limited clinical material

Table 3. Overview of computational and experimental systems pharmacology tools applied to deciphering ADC-induced hepatotoxicity, with key outputs and limitations. PBPK = physiologically based pharmacokinetic; QSAR = quantitative structure-activity relationship; scRNA-seq = single-cell RNA sequencing; DEG = differentially expressed genes; GO = gene ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes.

appropriate payload dose reduction, while necrotic injury implies a worse prognosis and different therapeutic management (Schomaker et al., 2013).For VOD-specific monitoring, angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF) serve as markers of sinusoidal endothelial activation and injury, with elevated Ang-2:Ang-1 ratios indicating LSEC destabilization that may precede overt VOD by days to weeks. These vascular biomarkers are particularly relevant for patients receiving inotuzumab ozogamicin or gemtuzumab ozogamicin, especially in the context of HSCT conditioning regimens (Richardson et al., 2017).

4.6 Bile Acid Panel and Transporter Biomarkers

The serum bile acid panel, measuring individual primary and secondary bile acids (cholic acid, chenodeoxycholic acid, deoxycholic acid, ursodeoxycholic acid, and their glycine/taurine conjugates), provides a sensitive and mechanistically informative index of bile acid transporter function. ADC-induced BSEP inhibition produces a characteristic pattern of elevated conjugated bile acid levels with preserved or reduced unconjugated fractions, distinguishable from hepatocellular injury patterns. Emerging proteomic approaches for quantifying circulating BSEP protein fragments may provide direct evidence of transporter protein degradation rather than functional inhibition alone, further improving mechanistic biomarker specificity (Mikkelsen et al., 2023).

4.7 High-Mobility Group Box 1 (HMGB1) and Inflammatory Biomarkers

HMGB1, a damage-associated molecular pattern (DAMP) released from hepatocytes undergoing necrosis, serves as a marker of sterile inflammatory activation in the liver. Unlike apoptotic cell death, necrotic hepatocyte death releases nuclear HMGB1 which, upon extracellular release, binds pattern recognition receptors (TLR4, RAGE) on Kupffer cells and LSECs, amplifying the inflammatory cascade. Elevated circulating HMGB1 in ADC-treated patients would indicate active hepatic necrosis and Kupffer cell activation, supporting the immunological amplification mechanism described in Section 4.5. While HMGB1 is not liver-specific, its combination with hepatocyte-specific markers (miR-122, GLDH) in a biomarker panel approach would provide both injury quantification and mechanistic pathway attribution.

Table 4. Biomarker Panel for ADC-Induced Hepatotoxicity: Mechanistic and Clinical Utility

Biomarker	Category	Mechanism of Elevation	ADC Context	Clinical Utility
ALT / AST	Traditional LFT	Hepatocyte membrane disruption by payload	T-DM1, gemtuzumab	First-line screening; limited specificity
Total bilirubin	Traditional LFT	Bile transporter inhibition or hemolysis	Inotuzumab, sacituzumab	VOD monitoring; cholestasis marker
ALP / GGT	Cholestatic markers	Bile duct/cholangiocyte injury; BSEP inhibition	Calicheamicin ADCs	Biliary toxicity; duct involvement
GLDH	Novel hepatocyte marker	Mitochondrial enzyme; specific to hepatocyte necrosis	T-DM1 preclinical data	Higher specificity than ALT for necrosis
miR-122	Circulating microRNA	Released specifically from hepatocytes	Emerging in ADC studies	Early, sensitive, liver-specific
High-mobility group box 1 (HMGB1)	Damage-associated molecular pattern	Released in pyroptosis/necrosis	Preclinical ADC models	Signals sterile inflammation

Plasma keratin-18 fragments (K18-Asp396)	Apoptosis biomarker	Caspase-cleavage product from hepatocyte apoptosis	Investigational	Distinguishes apoptosis vs necrosis
Angiopoietin-2 / VEGF	Vascular/sinusoidal	Endothelial damage; SOS/VOD indicator	Inotuzumab, gemtuzumab	VOD risk stratification
FGF21	Metabolic/hepatic stress	Stress response from hepatocytes	Exploratory	Mitochondrial dysfunction marker
BILE ACID PANEL	Cholestatic	Impaired hepatic extraction/conjugation	Sacituzumab, SN-38 ADCs	Sensitive BSEP inhibition indicator

Table 4. Proposed biomarker panel for comprehensive monitoring of ADC-induced hepatotoxicity, encompassing traditional and novel markers with mechanistic attribution. GLDH = glutamate dehydrogenase; HMGB1 = high-mobility group box 1; K18 = keratin-18; BSEP = bile salt export pump; FGF21 = fibroblast growth factor 21; LFT = liver function test; VOD = veno-occlusive disease.

5.0 BIOMARKERS FOR EARLY DETECTION AND MONITORING OF ADC-INDUCED HEPATOTOXICITY

5.1 Traditional Hepatic Biomarkers: Strengths and Limitations

The clinical monitoring of ADC-induced hepatotoxicity currently relies predominantly on traditional liver function tests (LFTs): serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and total bilirubin. These markers are graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE v5.0), with Grade 3 or 4 elevations triggering dose modification or discontinuation (National Cancer Institute, 2017). While universally available and well-validated for hepatocellular injury, traditional LFTs have significant limitations in the ADC context: they are not liver-specific (AST is elevated in muscle injury, hemolysis), they reflect injury that has already occurred rather than injury risk, they have poor sensitivity for early sinusoidal endothelial injury relevant to VOD, and they cannot distinguish the mechanistic basis of hepatotoxicity (hepatocellular vs cholestatic vs vascular) without additional clinical and pathological correlation (Mikkelsen et al., 2023).

5.2 Novel Circulating Biomarkers

Several novel biomarkers with superior hepatic specificity, mechanistic informativeness, and earlier injury detection capability have been investigated in the context of ADC hepatotoxicity. Circulating microRNA-122 (miR-122) is released specifically from hepatocytes upon membrane injury, demonstrating liver-tissue specificity exceeding that of ALT. In preclinical ADC toxicity studies and early clinical investigations, miR-122 elevations precede conventional transaminase elevation by 6–24 hours, and its magnitude correlates with the extent of hepatocellular necrosis—making it a promising early-warning marker for ADC dose escalation studies (Bhatt et al., 2020; Schomaker et al., 2013).Glutamate dehydrogenase (GLDH), a mitochondrial enzyme released specifically from hepatocyte necrosis, provides mechanistic information complementary to ALT: while ALT reflects membrane disruption from any cause (including apoptosis), GLDH elevation specifically indicates mitochondrial membrane compromise, enabling early identification of the mitochondrial dysfunction pathway relevant to maytansinoid and auristatin payloads. Keratin-18 (K18) fragmentation products generated by caspase-3 cleavage (K18-Asp396) distinguish apoptotic from necrotic hepatocyte death—a mechanistically important distinction because apoptotic hepatotoxicity may be reversible with

6.0 MITIGATION STRATEGIES AND THERAPEUTIC APPROACHES

6.1 ADC Engineering Strategies

The most impactful mitigation of hepatotoxicity lies in rational ADC design. Site-specific conjugation—placing payload molecules at defined positions on the antibody (e.g., engineered cysteine residues, unnatural amino acids, enzymatic conjugation sites)—eliminates the DAR heterogeneity inherent in conventional lysine

or reduced interchain cysteine conjugation. Uniform DAR ADCs exhibit more predictable pharmacokinetics, reduced high-DAR species (which aggregate and are preferentially cleared by Kupffer cells), and improved therapeutic index compared to heterogeneous DAR mixtures. The superior safety profile of trastuzumab deruxtecan (homogeneous DAR8 via enzyme-mediated conjugation) relative to earlier heterogeneous T-DM1 formulations partly reflects this design advantage (Junutula et al., 2008; Ogitani et al., 2016). Linker engineering towards greater plasma stability—through selection of more stable peptide sequences, incorporation of steric shielding around the cleavage site, or use of tumor-microenvironment-activated linkers that respond to stimuli unique to the tumor (hypoxia, matrix metalloproteinase activity, unique tumor-expressed enzymes)—represents a promising strategy for reducing hepatic free-payload exposure. The development of pH-responsive, microenvironment-activated cleavable linkers that are stable at physiological pH (7.4) but efficiently cleaved in the acidic tumor microenvironment (pH 6.5–6.8) without being activated by the mildly acidic hepatic lysosomal environment (pH ~5.5) requires precise linker chemistry optimization (Beck et al., 2017).

6.2 Masked and Activatable ADC Platforms

Masked ADCs (also termed Probodies or conditional ADCs) represent an advanced engineering strategy to improve the selectivity of antigen binding, thereby reducing on-target hepatotoxicity from low-level antigen expression on hepatobiliary cells. In this format, an inhibitory masking peptide blocks the antibody's antigen-binding domain (paratope), preventing target engagement in normal tissues. The mask is designed to be cleaved specifically by proteases—such as urokinase plasminogen activator (uPA) or matrix metalloprotease-2 (MMP-2)—that are highly expressed in the tumor microenvironment but minimally active in liver tissue. Unmasking within the tumor restores full antigen-binding activity, enabling targeted payload delivery, while the masked ADC circulates safely past normal hepatic cells expressing the same antigen at low levels (Maitland and Kasza, 2021).

6.3 Hepatoprotective Pharmacological Strategies

Ursodeoxycholic acid (UDCA), a hydrophilic bile acid with well-established hepatoprotective properties, has demonstrated efficacy in preventing ADC-induced cholestatic hepatotoxicity in preclinical mouse models through multiple mechanisms: competition with hydrophobic toxic bile acids for BSEP substrate binding, stimulation of bile flow via alternative bile acid export pathways (MRP4/ABCC4), and direct anti-apoptotic effects on hepatocytes via heat shock protein induction (Marschner et al., 2022). While clinical evidence for UDCA prophylaxis in the ADC setting is limited to small series and retrospective analyses, its excellent safety profile supports prospective investigation in patients receiving ADCs with known BSEP inhibitory payloads.For VOD/SOS, defibrotide—a polydisperse oligonucleotide with anti-thrombotic, anti-ischemic, and LSEC-protective properties—is the only FDA-approved treatment and has demonstrated survival benefit in severe VOD. Defibrotide acts through multiple complementary mechanisms: downregulation of endothelial adhesion molecules, enhancement of tissue plasminogen activator (tPA) activity, inhibition of heparanase-mediated extracellular matrix degradation, and direct cytoprotection of sinusoidal endothelial cells from cytotoxic injury (Richardson et al., 2017). Prophylactic defibrotide in high-risk patients receiving calicheamicin ADCs—particularly those with prior HSCT, hepatic comorbidities, or elevated baseline liver enzymes—is an area of active clinical investigation.N-acetylcysteine (NAC), a glutathione precursor with established efficacy in acetaminophen hepatotoxicity, has theoretical applicability to ADC-induced oxidative hepatotoxicity driven by GSH-depleting payload reactive intermediates. Preclinical data supporting NAC hepatoprotection in DM1-exposed hepatocyte models and its widespread clinical availability support its empirical use in severe ADC-related hepatotoxicity, though prospective controlled trials are needed. Fibroblast growth factor 21 (FGF21) analogs and mitochondria-targeted antioxidants (MitoQ, SS-31) represent experimental hepatoprotective strategies targeting the mitochondrial dysfunction pathway relevant to maytansinoid and auristatin payloads.

6.4 Dose Optimization and Therapeutic Drug Monitoring

Pharmacokinetic-pharmacodynamic (PK-PD) modeling has established clear exposure-response relationships between ADC payload exposure metrics (peak concentration Cmax, area under the curve AUC) and hepatotoxicity endpoints. Population PK analyses of T-DM1 in the EMILIA trial demonstratedthat patients in the highest quartile of T-DM1 AUC had 2.3-fold higher rates of Grade 3+ transaminase elevation compared to the lowest quartile, supporting individualized dose reduction strategies based on therapeutic drug monitoring (TDM) rather than fixed-dose reductions (Singh et al., 2020). Model-informed precision dosing (MIPD) algorithms that integrate individual patient PK parameters, hepatic function biomarkers, and tumor response data to optimize payload exposure within a defined therapeutic window represent an emerging approach to balancing

hepatotoxicity risk and efficacy.

6.5 Clinical Management Algorithms

The management of ADC-induced hepatotoxicity in clinical practice follows CTCAE-based grading algorithms that recommend dose interruption for Grade 2 elevations, dose reduction for Grade 3, and permanent discontinuation for Grade 4 or for any Grade associated with clinical symptoms of liver failure (National Cancer Institute, 2017; Oken et al., 1982). These algorithms were developed primarily based on experience with conventional cytotoxic chemotherapy and may not fully capture the distinct temporal kinetics and mechanistic patterns of ADC hepatotoxicity. Specialized ADC hepatotoxicity management algorithms that incorporate novel biomarker monitoring, hepatic imaging (Doppler ultrasound for VOD), liver biopsy indications, and mechanism-specific management strategies (e.g., defibrotide for VOD, UDCA for cholestasis) are needed to optimize patient outcomes (Hoffman et al., 2021).

Table 5. Mitigation Strategies for ADC-Induced Hepatotoxicity

Strategy	Mechanism	Supporting Evidence	Stage of Development
Site-specific conjugation (DAR control)	Reduces heterogeneity; limits excess payload causing off-target liver uptake	T-DXd vs T-DM1 comparative hepatotoxicity data	Approved (T-DXd); broad adoption ongoing
Linker engineering (stable linkers)	Prevents premature payload release in circulation; reduces free-drug hepatic exposure	Revised gemtuzumab formulation (2017); reduced VOD rate	Approved; active linker optimization programs
Payload modification (membrane-impermeable)	Limits bystander toxicity in liver; MMAF vs MMAE differential hepatic entry	Belantamab data; in vitro hepatocyte studies	Clinical use (belantamab); investigational
Defibrotide prophylaxis	Protects sinusoidal endothelium; reduces VOD/SOS in high-risk patients	Phase III REACH trial; FDA-approved for VOD	Approved for VOD treatment; prophylaxis trials ongoing
Dose optimization / scheduling	Fractionated dosing reduces peak hepatic payload concentration	PK-PD modeling studies; T-DM1 fractionation data	Investigational; clinical PK studies
Ursodeoxycholic acid (UDCA)	Hepatoprotective bile acid; displaces toxic bile acids; BSEP protective	Preclinical cholestasis models; pilot studies	Investigational; small clinical series
N-acetylcysteine (NAC)	Replenishes GSH; reduces oxidative payload-mediated hepatocyte injury	In vitro data; DILI treatment evidence	Investigational; used empirically in severe DILI
FKBP51 / NF-kB pathway inhibition	Reduces Kupffer cell-mediated inflammatory amplification	Preclinical macrophage depletion models	Early preclinical
Hepatocyte-sparing ADC design (masked ADCs)	Masking antibody until tumor microenvironment activation; reduces off-target liver binding	Probody platform (CytomX); PBPK validation	Phase I/II clinical trials
Therapeutic drug monitoring (TDM)	Individualize dosing based on payload PK; avoid hepatotoxic trough/peak levels	Exposure-response analyses; ASCO guidelines	Investigational; emerging practice

Table 5. Evidence-based and investigational strategies for mitigation of ADC-induced hepatotoxicity, spanning ADC engineering, pharmacological hepatoprotection, dose optimization, and next-generation ADC design. DAR = drug-to-

antibody ratio; VOD = veno-occlusive disease; BSEP = bile salt export pump; UDCA = ursodeoxycholic acid; NAC = N-acetylcysteine; MIPD = model-informed precision dosing.

7.0 REGULATORY PERSPECTIVES AND FUTURE DIRECTIONS

7.1 FDA and EMA Regulatory Framework for ADC Hepatotoxicity

Regulatory agencies have developed evolving frameworks for addressing hepatotoxicity in ADC development programs. The FDA's 2023 Guidance for Industry on Drug-Induced Liver Injury (DILI) provides overarching principles for hepatotoxicity evaluation in oncology drug development, including the requirement for robust LFT monitoring in clinical trials, hepatic impairment pharmacokinetic studies, and Hy's Law evaluation (FDA, 2023). However, the unique pharmacological properties of ADCs—including the multi-component structure, the payload as the primary cytotoxic agent, and the mechanistic complexity of on-target versus off-target hepatotoxicity—have prompted calls for ADC-specific regulatory guidance.The EMA's 2022 guideline on antibody-drug conjugate toxicity investigation recommends that ADC development programs include dedicated mechanistic hepatotoxicity studies, incorporating in vitro hepatocyte and LSEC models, PBPK modeling to predict hepatic payload exposure, and exploratory novel biomarker assessment to complement traditional LFT monitoring in early clinical trials (EMA, 2022). Both agencies have issued black box warnings for hepatic VOD with calicheamicin-bearing ADCs and require mandatory monitoring, dose modification, and VOD management protocols in package inserts (Saber and Leighton, 2015).The FDA's Oncology Center of Excellence has identified ADC hepatotoxicity as a priority area for regulatory science research, with ongoing collaborations with academic institutions and industry partners to develop consensus biomarker qualification pathways for novel hepatotoxicity markers (miR-122, GLDH, K18 fragments) and to establish standardized in vitro test battery recommendations for ADC hepatotoxicity preclinical assessment (Hoffman et al., 2021).

7.2 Challenges in Translational Prediction

A fundamental challenge in translating preclinical ADC hepatotoxicity findings to clinical risk prediction is the substantial interspecies difference in key determinants of ADC hepatic exposure: FcRn expression and affinity, Fcγ receptor distribution on hepatic macrophages, cathepsin B activity in hepatic lysosomes, bile acid transporter substrate specificity, and CYP3A4 metabolic capacity. Standard rodent models substantially underpredict ADC-induced hepatotoxicity due to lower FcRn-mediated ADC half-life (and thus lower cumulative hepatic exposure), different Kupffer cell Fcγreceptor repertoires, and lack of cross-reactivity for human-specific targets (CD33, HER2) expressed on murine hepatic cells (Stagg et al., 2016).Non-human primate (NHP) models, particularly cynomolgus macaques, provide substantially better translational fidelity for ADC hepatotoxicity due to their closer phylogenetic relationship with human hepatic physiology. However, the expense, ethical constraints, and limited throughput of NHP studies preclude their use for early-stage ADC screening. Humanized mouse models—incorporating human FcRn, human Fcγ receptors, or human-relevant antigen expression—represent promising intermediate solutions, enabling more human-relevant ADC hepatic pharmacokinetics in a rodent platform (Saber and Leighton, 2015).

7.3 Future Research Priorities

Several key research priorities will shape the field of ADC hepatotoxicity over the coming decade. First, the development and clinical qualification of novel hepatotoxicity biomarkers (miR-122, GLDH, K18 fragments, Ang-2, bile acid panels) through prospective correlative studies in ADC clinical trials will enable evidence-based replacement of traditional LFT monitoring with more sensitive and mechanistically informative panels. Second, the integration of artificial intelligence and machine learning with multi-omics and clinical data will enable development of predictive hepatotoxicity classifiers that can identify high-risk patients and ADC designs prior to clinical evaluation (Rodrigues et al., 2019).Third, the systematic application of QSP

frameworks—combining PBPK modeling, mechanistic toxicity modeling, and clinical PK-PD analysis—will enable model-informed precision dosing strategies that individualize ADC therapy based on patient-specific hepatic function, PK parameters, and biomarker profiles. Fourth, the development of standardized, mechanistically comprehensive in vitro hepatic test batteries incorporating 3D organoids, liver-on-a-chip systems, and immune co-cultures will provide higher-fidelity preclinical hepatotoxicity assessment that reduces late-stage clinical attrition due to unexpected hepatic adverse events. Fifth, the exploration of novel hepatoprotective strategies—including Nrf2 activators, mitochondria-targeted antioxidants, farnesoid X receptor (FXR) agonists for BSEP upregulation, and epigenetic modifiers—may yield combination approaches that preserve ADC antitumor efficacy while substantially reducing hepatic toxicity burden (Marschner et al., 2022).

CONCLUSION

ADC-induced hepatotoxicity represents a complex, multifactorial adverse event landscape shaped by the intersection of antibody pharmacokinetics, linker chemistry, payload biology, and hepatic physiology. As the ADC field rapidly expands—with novel payloads, engineered linkers, bispecific formats, and immune-stimulating ADCs entering development—the mechanistic complexity of hepatotoxicity is expected to increase commensurately. The on-target and off-target mechanisms reviewed here are not mutually exclusive; in clinical practice, multiple mechanisms often operate simultaneously and synergistically in individual patients, influenced by tumor biology, hepatic comorbidities, concomitant medications, and genetic polymorphisms in drug-metabolizing enzymes and bile acid transporters.A systems pharmacology approach—integrating PBPK modeling, network pharmacology, molecular docking, multi-omics profiling, and advanced hepatic model systems within a coherent quantitative framework—offers the most powerful strategy currently available for deciphering the mechanistic complexity of ADC-induced hepatotoxicity. The translation of mechanistic insights into actionable clinical tools (novel biomarker panels, TDM algorithms, mechanism-specific management guidelines, PBPK-informed dose reduction recommendations) will require sustained collaborative effort between computational scientists, hepatologists, clinical pharmacologists, and regulatory scientists.The ultimate goal—improving patient outcomes by maximizing the antitumor efficacy of ADCs while minimizing hepatic adverse events through rational design and individualized management—is achievable through the integration of engineering innovation, mechanistic science, and clinical translation that characterizes the systems pharmacology paradigm. The lessons learned from the hepatotoxicity challenges of current-generation ADCs must be prospectively applied to the design and development of next-generation conjugates, ensuring that therapeutic index optimization is a foundational design criterion rather than a post-hoc safety concern.

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DECLARATION OF INTEREST

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