Industry Clinical Perspective on DILI and CIVM Application

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Historical perspective

- Hy Zimmerman MD, lecturing on the mechanisms of DILI (c. 1970)

Lewis et al. AASLD, 2020
Current challenges posed by DILI in drug development

• A major cause of **early termination** of drug development (1)

• A frequent cause for **regulatory action** and limiting drug use

• Most cases are **idiosyncratic** and therefore **difficult** or **impossible to predict**

• **Lack of biomarkers** for risk assessment, diagnosis, and outcome prediction

• **Limited translatability** of nonclinical findings

(1) Guengerich F. *Drug Metab Pharmacokinet.* 2010
Objectives

• Industry perspective on human DILI

• Highlight impact of CIVM on IND submission
Patient A.

- Presented with ALT 3 x ULN, Phase 1 SAD
Patient B.

• Presented with elevated ALT, fever and rash, Phase 3
Patient C.

- Presented with elevated ALT and ANA(+), post-approval
## Phenotypes of human DILI

<table>
<thead>
<tr>
<th>Acute hepatic necrosis</th>
<th>Acute hepatitis</th>
<th>Bland cholestasis/ Mixed cholestatic hepatitis</th>
<th>Vanishing bile duct syndrome</th>
<th>Hypersensitivity syndrome with liver involvement (DRESS)</th>
</tr>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<td><img src="image5.png" alt="Image" /></td>
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<table>
<thead>
<tr>
<th>Severe cutaneous adverse reactions (SJS/TEN)</th>
<th>Drug-induced autoimmune hepatitis</th>
<th>Hepatic steatosis (macrosteatosis)</th>
<th>Acute fatty liver (microsteatosis)</th>
<th>Sinusoidal obstruction syndrome</th>
</tr>
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<tr>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
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<td><img src="image10.png" alt="Image" /></td>
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<tr>
<th>Nodular regenerative hyperplasia</th>
<th>Neoplasia</th>
<th>Secondary sclerosing cholangitis</th>
<th>Granulomatous hepatitis</th>
<th>Peliosis hepatitis</th>
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<tbody>
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<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
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<td><img src="image14.png" alt="Image" /></td>
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Multiple facets of human DILI

• 3 major **categories** (direct, idiosyncratic, indirect)

• 11+ potential **mechanisms** (1)

• 15+ **phenotypes** (2)

• 3 **biochemical patterns** (hepatocellular, cholestatic, mixed)

• 4+ routine **diagnostic biomarkers** (ALT, AST, alkaline phosphatase, bilirubin)

(1) Hoofnagle et al. NEJM, 2019
(2) CIOMS Working Group, 2020
CIVM and categories of DILI

- CIVM is well-suited to detect **direct** hepatotoxicity
  - Lead optimization (i.e., candidate selection)
  - Due diligence (collaborations, acquisitions)
  - Preclinical
  - Phase 1

- CIVM can fill a **key unmet need** with **idiosyncratic** hepatotoxicity
  - Limitations with animal models
  - Low frequency of severe DILI in development programs/registrational trials
  - Opportunity to screen (e.g., human donor genotypic variability)
  - Drug-drug interactions

- CIVM and **indirect** hepatotoxicity may be considered ‘**future direction**’
CIVM and potential mechanisms of DILI

- Various mechanisms have been proposed

  - Formation of reactive metabolites
  - Hapten formation
  - Danger signals hypothesis
  - Bile Salt Export Pump (BSEP) inhibition
  - Drug transporter/metabolizing enzymes modulation
  - Mitochondrial toxicity
  - Oxidative stress
  - Modulating adaptive/innate immune reactions
  - Biliary epithelial injury
  - Histone acetylation
  - Autoimmunity
  - Immune-mediated toxicity

Luedde T et al. Gastroenterology 2014;147:765-83
Can CIVM expand breadth of diagnostic biomarkers?

- **Glycochenodeoxycholic acid** (GCDCA) as a surrogate biomarker of cholestasis
- **Urea** synthesis as surrogate biomarker of mitochondrial function
- **miR122**, GLDH and **cytokines** as biomarkers of hepatocyte damage
- **Autoantibodies** and **Th17** as biomarkers of autoimmunity
- **DAMPs** and **macrophage phenotypes** as biomarkers of immune stimulation
How can CIVM impact IND submission

• As part of a nonclinical toxicology package

• Inform ‘pre-test probability’ of DILI

• Support rationale for conducting/not-conducting Phase 1 liver safety study

• Impact design of Phase 2/3 studies (e.g., screening labs; frequency of surveillance exclusion criteria; discontinuation rules)

• Is there a role for CIVM and prediction of adaptation (for development programs with imbalance of cases in Temple’s quadrant but not Hy’s quadrant)
A hepatologist sees hepatology everywhere
Patients A, B and C.

- Which cell types and biomarkers will CIVM incorporate, to define immunologic DILI pathways? Update this sentence

Adams D et al. *Tox Sciences*, 2010
(Macro) Anatomy

- Hepatic parenchyma
- Biliary structures
- Vasculature
**In vivo microanatomy**

- **Portal Triad (Zone 1):**
  - Portal vein (P)
  - Hepatic artery
  - Bile duct

- Blood flow: 1 → 2 → 3

- Implications for *in vitro* modeling
Phenotype

Phenotype is defined as the state of an organism resulting from interactions between genes, environment, disease, molecular mechanisms, and chance.

From: Advances in Genetics, 2016

“DILI Phenotype” - the *observable* patterns/syndromes associated with a given drug injury
Acute hepatic necrosis

- Zone 3 (*) is notable for being oxygen-poor and metabolizing enzyme-rich
- Leading cause of acute liver failure (ALF) in the US
Acute hepatic necrosis

- Zone 3 (*) is notable for being oxygen-poor and metabolizing enzyme-rich
- Leading cause of acute liver failure (ALF) in the US
- Acetaminophen, aspirin, sunitinib, MTX, regorafenib, brentuximab
Acute hepatitis

- Classic ‘idiosyncratic’ and immune-mediated injury
- “Hepatocellular jaundice” as described in Hy’s law
- Symptoms, histology resemble viral hepatitis
- *In vitro* Biomarker priority
Risk assessment for severe liver injury

Hy’s Law: Predicts Impaired Hepatic Function

After Antoine et al, March 2015
Bland cholestasis and Mixed (cholestatic) hepatitis

- “Cholestasis” – defective movement of bilirubin or bile; can occur at 3 levels
- May account for almost 50% of all DILI
- Better prognosis (vs acute hepatitis)
Hypersensitivity syndrome with liver involvement (DRESS)

- Life-threatening disease (mortality = 10%) with cutaneous presentation and internal organ involvement (liver > 50%)
- Latency 2-8 weeks
- Relapses/flares are common, even after drug discontinuation
- Biomarker: eosinophils (?)
Severe cutaneous adverse reactions

- SJS/TEN: High mortality that increases in the presence of DILI (36-46%); positive rechallenge is common

- Mostly cholestatic injury

- C
Drug-induced autoimmune hepatitis

- Mimics regular AIH serology, histology but response to steroids is different (i.e. does not relapse)
- Plasma cells (arrows) secrete immunoglobulins (e.g. IgG)
- Similar histology seen in ILICI (checkpoint inhibitors)
- Vs Drug as environmental antigen
Hepatic steatosis

- Steatosis has a broad differential diagnosis:
  - NASH
  - Alcohol
  - Wilson’s disease
  - HCV GT3
  - Parenteral nutrition
  - Starvation
  - Acute fatty liver of pregnancy
  - Drugs

- M
Acute fatty liver

- Acute onset of microvesicular steatosis

- Altered mitochondrial respiration, reduced beta-oxidation leads to triglyceride accumulation
Sinusoidal obstruction syndrome (SOS; VOD)

- Injury is to the sinusoidal epithelium, causing sloughing and microthrombi

- Sequelae from incr. pressure:
  - hepatomegaly
  - RUQ pain
  - Ascites (weight gain)

- Myeloablative agents
Nodular regenerative hyperplasia (NRH)

- A regenerative response (micronodular transformation) to vascular injury
- Common cause of *non*-cirrhotic portal hypertension
- AZA, MTX, Vitamin A
Neoplasia

- Drugs encourage tumor formation:
  - adenoma
  - hepatocellular carcinoma

- Biopsy: too many hepatocytes in between each sinusoid

- Oral contraceptives, androgens
Secondary sclerosing cholangitis

• Insult to biliary tree triggers a fibro-inflammatory reaction

• Can cause jaundice, intense pruritus

• Amoxicillin-clavulanate, infliximab, sevoflurane
Granulomatous hepatitis

- Inflammatory reaction to noxious stimuli; macrophages, lymphocytes infiltrate hepatic lobule (Zone 2)

- Phenytoin, sulfa drugs, herbals
Peliosis hepatis

- Rupture of dilated sinusoids causes formation of large, blood-filled spaces

- Steroids, MTX, 6MP, tamoxifen
Vanishing bile duct syndrome

- typically occurs after a bout of severe cholestatic hepatitis, often with immunoallergic features

- AZA, erythromycin, amoxicillin-clavulanate
Parting thoughts: Particularities that apply to hepatotoxicity in the oncology setting

- Malnutrition, polypharmacy may increase susceptibility

- Differential diagnosis
  - liver metastases
  - bile duct obstruction
  - bone metastases
  - heart failure
  - sepsis
  - reactivation of viral hepatitis

- Don’t forget about con meds: herbals and CAM

- High dose or daily therapy
Conclusions

• DILI is complicated.

• (Un)fortunately, AST/ALT/AP/Bil are the lone biomarkers; they suffer from poor specificity and positive predictive value.

• For the astute oncologic drug developer, an understanding of the fifteen phenotypes of DILI may prove useful.
The liver in context

- produce bile for fat digestion
- maintain coagulation homeostasis
- synthesize/store glucose
- maintain iron homeostasis
- synthesize cholesterol
- synthesize proteins
- stores vitamins and minerals
- metabolize drugs
Levels of DILI Severity

5  Death or Tx
4  Acute Liver Failure
3  Serious: Disabled, Hospitalized
2  Hy’s Case: Injury with Slight Functional Loss
1  Serum Enzyme Elevations Only; Many People Adapt
Biomarkers of Liver Safety

- When there is injury to the hepatocytes
  - Aspartate aminotransferase (AST)
  - Alanine aminotransferase (ALT)

Healthy hepatocytes (pink); sinusoids (white)
Biomarkers of Liver Safety

- When there is injury to the bile ducts
  - alkaline phosphatase (AP)
- What about bilirubin?
  - elevated bilirubin can be from
    - biliary disease (e.g. obstruction)
    - hepatocyte injury (e.g. Hy’s Law)
    - liver insufficiency (e.g. ESLD)
## Three main causes of DILI (Direct, Idiosyncratic, Indirect)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Direct Hepatotoxicity</th>
<th>Idiosyncratic Hepatotoxicity</th>
<th>Indirect Hepatotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Common</td>
<td>Rare</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Dose-related</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Predictable</td>
<td>Yes</td>
<td>No</td>
<td>Partially</td>
</tr>
<tr>
<td>Reproducible in animal models</td>
<td>Yes</td>
<td>No</td>
<td>Not usually</td>
</tr>
<tr>
<td>Latency (time to onset)</td>
<td>Typically rapid (days)</td>
<td>Variable (days to years)</td>
<td>Delayed (months)</td>
</tr>
<tr>
<td>Phenotypes</td>
<td>Acute hepatic necrosis, serum enzyme elevations, sinusoidal obstruction, acute fatty liver, nodular regeneration</td>
<td>Acute hepatocellular hepatitis, mixed or cholestatic hepatitis, bland cholestasis, chronic hepatitis</td>
<td>Acute hepatitis, immune-mediated hepatitis, fatty liver, chronic hepatitis</td>
</tr>
<tr>
<td>Most commonly implicated agents</td>
<td>High doses of acetaminophen, niacin, aspirin, cocaine, IV amiodarone, IV methotrexate, cancer chemotherapy</td>
<td>Amoxicillin–clavulanate, cephalosporins, isoniazid, nitrofurantoin, minocycline, fluoroquinolones, macrolide antibiotics</td>
<td>Antineoplastic agents, glucocorticoids, monoclonal antibodies (against tumor necrosis factor, CD20, checkpoint proteins), protein kinase inhibitors</td>
</tr>
<tr>
<td>Cause</td>
<td>Intrinsic hepatotoxicity when agent given in high doses</td>
<td>Idiosyncratic metabolic or immunologic reaction</td>
<td>Indirect action of agent on liver or immune system</td>
</tr>
</tbody>
</table>

* IV denotes intravenous.
## Eleven Phenotypes of DILI

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Type of Liver Injury</th>
<th>Latency</th>
<th>Enzyme Pattern</th>
<th>Typical Agents</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute hepatic necrosis</td>
<td>Direct</td>
<td>Days</td>
<td>Marked, abrupt ALT elevations; mild Alk P and bilirubin elevations</td>
<td>Acetaminophen, aspirin, niacin, “Ecstasy”</td>
<td>Often due to overdose</td>
</tr>
<tr>
<td>Enzyme elevations</td>
<td>Direct</td>
<td>Days to months</td>
<td>Mild-to-moderate ALT or Alk P elevations</td>
<td>Isoniazid, diclofenac</td>
<td>Usually transient and asymptomatic</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>Idiosyncratic, indirect</td>
<td>Days to months</td>
<td>High ALT elevations, modest Alk P elevations</td>
<td>Amoxicillin–clavulinate, cefazolin</td>
<td>High death rate</td>
</tr>
<tr>
<td>Cholestatic hepatitis</td>
<td>Idiosyncratic</td>
<td>Weeks to months</td>
<td>High Alk P elevations, modest ALT elevations</td>
<td>Prunitus, early and prominent</td>
<td></td>
</tr>
<tr>
<td>Mixed hepatitis</td>
<td>Idiosyncratic</td>
<td>Days to months</td>
<td>Moderate Alk and Alk P elevations</td>
<td>TMP-SMZ, phenytoin</td>
<td>Usually benign, self-limited</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>Idiosyncratic, indirect</td>
<td>Months to years</td>
<td>Moderate ALT elevations with bilirubin elevations</td>
<td>Diclofenac, nitrofurantoin, minocycline</td>
<td>Insidious onset; may require glucocorticoids</td>
</tr>
<tr>
<td>Bland cholestasis</td>
<td>Unknown, possibly idiosyncratic</td>
<td>Months</td>
<td>Moderate ALT elevations, mild Alk P elevations</td>
<td>Anabolic steroids, estrogens</td>
<td>Prunitus, prominent and prolonged</td>
</tr>
<tr>
<td>Acute fatty liver, lactic acidosis, and hepatic failure</td>
<td>Direct</td>
<td>Days to months</td>
<td>Lactic acidosis, modest ALT elevations, hepatic failure</td>
<td>Stavudine, linezolid, aspirin (Reye’s syndrome)</td>
<td>Mitochondrial failure, pancreatitis</td>
</tr>
<tr>
<td>Nonalcoholic fatty liver</td>
<td>Indirect, direct</td>
<td>Months</td>
<td>Mild ALT and Alk P elevations</td>
<td>Glucocorticoids, tamoxifen, haloperidol</td>
<td>Asymptomatic; fatty liver seen on ultrasound</td>
</tr>
<tr>
<td>Sinusoidal obstruction syndrome</td>
<td>Direct</td>
<td>Weeks</td>
<td>Variable enzyme elevations</td>
<td>Cancer agents, busulfan, gemtuzumab</td>
<td>Hepatomegaly, weight gain, edema, ascites</td>
</tr>
<tr>
<td>Nodular regenerative hyperplasia</td>
<td>Direct</td>
<td>Years</td>
<td>Minimal ALT and Alk P elevations</td>
<td>Thiouganine, azathioprine, oxaliplatin</td>
<td>Noncirrhotic portal hypertension</td>
</tr>
</tbody>
</table>

* The phenotypes are listed very generally in order of frequency; there is some overlap between idiosyncratic and indirect forms of injury. Alk P denotes alkaline phosphatase, ALT alanine aminotransferase, and TMP-SMZ trimethoprim–sulfamethoxazole.
Fibrosis progression, in 4 stages

**Portal fibrosis**

**Periportal fibrosis**

**A**

**B**

**C**

**D**

Bridging fibrosis

Cirrhosis