Finding Biomarkers to Predict and Manage Serious Drug-Induced Liver Injury: *Where to Look*

John C Bloom, VMD, PhD
Diagnostic and Experimental Medicine
Eli Lilly & Company

Detecting and Investigating Drug-Induced Liver Injury During Clinical Trials

Co-Sponsored by: Food and Drug Administration/Center for Drug Evaluation and Research (FDA/CDER), the Pharmaceutical Research and Manufacturers of America (PhRMA), and the American Association for the Study of Liver Disease (AASLD). 26 – 27 March, 2008, Silver Spring, MD

DR. BLOOM: I want to express my thanks to John Pears and Lana Pauls and John Senior, as with Dr. Seeff, a qualified thanks to John Senior for assigning me a no-win assignment. The assignment was how can we find biomarkers for DILI in clinical trials? I bastardized that a bit in terms of finding biomarkers to predict and manage serious drug-induced liver injury: where to look. By the way, I'm the poor orphan as far as slides. They're black and white and there's no animation. John just muttered no content. (Laughter.) I had a Lilly logo here that -- there's a logo police at Lilly and they have a logo that says Lilly answers the matter which I bastardized to markers that answer, but I took that off this presentation because I don't have markers to answer.

Biomarkers and Related Tools to Predict and Manage DILI: *Unmet Needs*

- Identification of candidate drugs with this potential.
- Identification of patients at risk or predisposed.
- Early detection and management of affected patients in clinical trials and practice.

Fortunately Mark and others have nicely gone through the preliminary slides that I've provided here as far as the unmet needs, the identification of the candidate drugs, patients are at risk and, of course, early detection and management of the affected patients in practice,

Finding Biomarkers that Enable DILI Risk Assessment and Management

- Discovery and validation of novel clinical biomarkers.
- New applications of established markers.

and that these, of course, include both how we use established markers and truly novel clinical biomarkers. Certainly the Hy's Law, if you will, uses established markers in a way that has provided one of the only truly predictive markers for DILI that can be employed in clinical development.

Guidance for Industry

Drug-Induced Liver Injury: Premarketing Clinical Evaluation

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact (CDER) Ruyi He at 301-796-0910, (CDER) Thomas Moreno at 301-796-2247, or (CBER) Bruce Schneider at 301-827-8343.

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)
October 2007
Drug Safety

I'm also going to be working significantly off the Guidance document in terms of context, and with that, I'm going to be framing, rather than throwing a lot of literature and data at you, what I regard as the defining questions, questions that define the possible.

DILI Biomarker Discovery:
Questions that define the possible

1. Working definition of DILI

   (as suggested in Draft Guidance document)

   - Severe, life-threatening, principally hepatocellular drug-induced injury
   - Rare (< 1/1000, most < 1/10,000)
   - Depends on individual susceptibilities that to date have not been characterized
   - Distinct from predictable, dose-related hepatotoxicity

1a. Does DILI represent a continuum of disorders regarding incidence (eg 1/50 to $1/10^6$)?

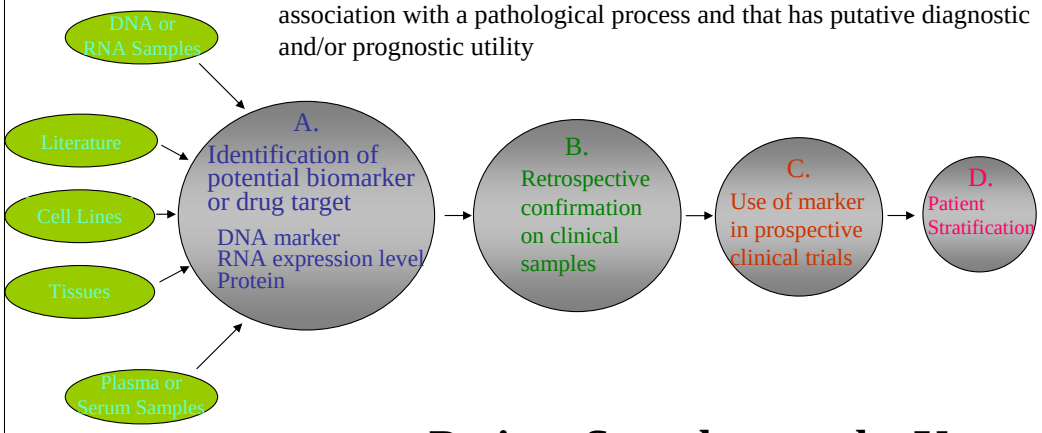
We have alluded to how we're defining DILI, and again I'm working off the Guidance document for the purpose of this discussion that includes severe, life-threatening, principally hepatocellular drug-induced injury, that is rare. It depends on individual susceptibilities that have not yet been characterized and is distinct from the predictable, dose-related hepatotoxity. I kept dose-related in there to annoy Jack Uetrecht. Obviously that's debatable.

And so a sub-question to that is does DILI represent a continuum of disorders regarding incidence? Certainly if we're considering things like isoniazid, some of the eloquent work recently with the -- even though that was looking into broader hypersensitivity adverse effect, we're talking numbers that make it feasible to start to detect and characterize and develop markers in the premarketing arena but, of course, that's going to depend significantly on incidence in my view, and we'll come back to that issue.

Biomarkers in drug development

Development of a biomarker

Biomarker: A physiological response or laboratory test that occurs in association with a pathological process and that has putative diagnostic and/or prognostic utility



Patient Samples are the Key

The problem of accessing the relevant patients relates to how we traditionally develop biomarkers. In drug development, we have a variety of sources as far as the literature. We rely very heavily on specimens, biological specimens, so that we can collect and acquire various cell lines, et cetera, et cetera, and we identify potential biomarker for a drug target. It might be DNA or RNA expression level, protein. We conduct a retrospective confirmation on clinical trails and we use the marker in prospective studies often for patients stratification or patient exclusion as it relates to risk and, of course, that's what we would very much like to do with DILI but we obviously can't use that approach that's here because we don't have access to the patients and the samples to achieve that.

DILI Biomarker Discovery:
Questions that define the possible (Con't)

2. Do candidate drugs that show demonstrable hepatotoxic signals premarketing (short of Hy's Law) pose a greater threat of DILI post-marketing than those that do not?

a. Is there a subset of clinical trial subjects showing heptotoxicity signals that are predisposed to DILI?

b. Do DILI (as defined) and hepatotoxicity more readily identified premarketing share mechanisms, risk factors, diagnostic/management challenges and, accordingly, biomarker discovery opportunities?

c. Can a proven DILI agent show hepatotoxicity signals in animals or man (retrospectively or prospectively) for which there are distinctively different mechanisms and risks?

There're a couple of other questions that I think are defining. One is do candidate drugs that show demonstrable hepatotoxic signals premarketing, and I'm excluding Hy's Law here, for the reasons we discussed, pose a greater treat of DILI post-marketing than those that do not?

Now that doesn't mean that understanding what the mechanistic of hepatotoxicity can be very important in helping manage what emerges post-marketing, if, for example, it's indeed dependent on the 1 in 10,000 susceptibility of a patient, that we discover post-marketing to what extent is this kind of toxicity relevant?

And related is there a subset of clinical trial subjects showing hepatotoxicity signals that are predisposed to DILI? We've had some discussions on that. If so, how do you find them? You just study the whole root. You study the whole root prospectively. I know John and others have suggested that when we forward this to identify drugs that have hepatotoxic potential that are going to be developed anyhow in the context of risk benefit, and longitudinally study them and acquire a deep knowledge and relate the biomarkers as to the reactions and that might be a way forward. But again, it depends on whether it's an issue of susceptibility rather than the mechanisms of the putative drug-induced toxicity.

Do DILI, as defined, and hepatotoxicity, other hepatotoxicity, more readily identify premarketing share mechanisms, risk factors, diagnostic/management challenges and, accordingly, biomarker discovery opportunities? That's obviously key regarding where we look.

And, can a proven DILI agent show hepatotoxicity signals in animals or man, either prospectively or retrospectively, for which there are distinctly different mechanisms and risks? When we go back and say, ah-ha, we have a signal in clinical development and we just haven't acquired enough or we take a putative agent back and run the drill again and say, well, we're now able to show some signals, is it the same disease? Does it have the same mechanism?

DILI Biomarker Discovery:
Questions that define the possible (Con't)

3. If the risk of DILI is indeed based on "individual susceptibilities", are those predisposing factors candidate/agent specific?
   - Generation of a toxic metabolite
   - Demonstrable a hypersensitivity reaction
   - Ability to compensate or adapt to liver injury
   - Genetic/molecular markers for the above

If the risk of DILI is indeed based on individual susceptibilities, are those predisposing factors candidate or agent specific? That's important with the DILI Network, for example, where we're pulling a lot of specimens from different candidate induced disease and we're looking for common markers. And as regards generation of a toxic metabolite, demonstrable hypersensitivity reaction, ability to compensate or adapt to liver injury or genetic or molecular markers for the above, John readily admitted that with the Isoniazid proposal, that that is very important to establish markers and understand what's going on there because the importance of that prospectively. Whether or not that has any value to other candidate drugs is questionable but as he mentions, that sets the goal standard or form a mile precedent as to the possible and the value of prospective studies like that.

DILI Biomarker Discovery:
Questions that define the possible (Con't)

4. In the context of the above, is it reasonable to suspect that premarketing observations beyond Hy's Law can have predictive value for DILI ?

*"Retrospective evaluation of earlier experiences, augmented by recent experience, lead us to believe that appropriate testing and analysis in premarketing studies may improve the early **detection** of drugs that can cause severe hepatocellular injury."*
(Draft Guidance Document, P 3)

In the context of what I just reviewed then, is it reasonable to suspect that premarketing observations beyond Hy's Law can have predictive value for DILI? Well, the Guidance document suggests it can, and an issue is whether they're talking about more than Hy's Law, that is to say, retrospective evaluation of earlier experiences, augmented by recent experience, lead us to believe that appropriate testing and analysis in premarketing studies may improve the early detection of drugs that can cause severe hepatocellular toxicity. And we would need to consider what the data to underpin that are beyond Hy's Law.

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
  -unprecedented targets and chemical entities
- New uses for established markers
- Genetic/molecular profiling

So if we accept that we may not be looking at the right populations but yet there's a family of biomarkers that we can consider that are applicable to the management of hepatotoxicity of which DILI might be embedded in there somewhere, I've listed categories here. I'm not going to go through these in any great lengths.

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
  -unprecedented targets and chemical entities
- New uses for established markers
- Genetic/molecular profiling

There's well-established risk factors, in-vitro and in silico models, mechanism-based screening, clinico-pathological profiles, the agent-specific signatures that keeps coming up that we see and how that relates to unprecedented targets and chemical entities is obviously problematic. New uses for established markers and then genetic and molecular profiling that Arthur will be going into in greater detail.

Risk Factors as Markers for DILI Susceptibility

Host factors:
race, age, sex, pre-existing liver disease, genetic factors, comorbidities, nutrition, body mass, renal function, etc

Agent factors:
structural alerts, covalent binding, glutathione depletion/drug or metabolite conjugates, drug interactions*, bioaccumulation in the liver, toxicity gene induction, P450 enzyme induction, preclinical hepatotox findings at steady state concentration in liver without safety margin

* eg induction of CYP2E1 by isoniazid ↑ susceptibility to acetaminophen toxicity

There's a host of risk factors here. I'm not going through it. Some of them are agent factors in terms of structural alerts, covalent binding, glutathione depletion/drug or metabolite conjugates, drug interactions, such as we have with the isoniazid and acetaminophen, P450 enzyme induction, et cetera, et cetera. So these are markers.

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
  -unprecedented targets and chemical entities
- New uses for established markers
- Genetic/molecular profiling

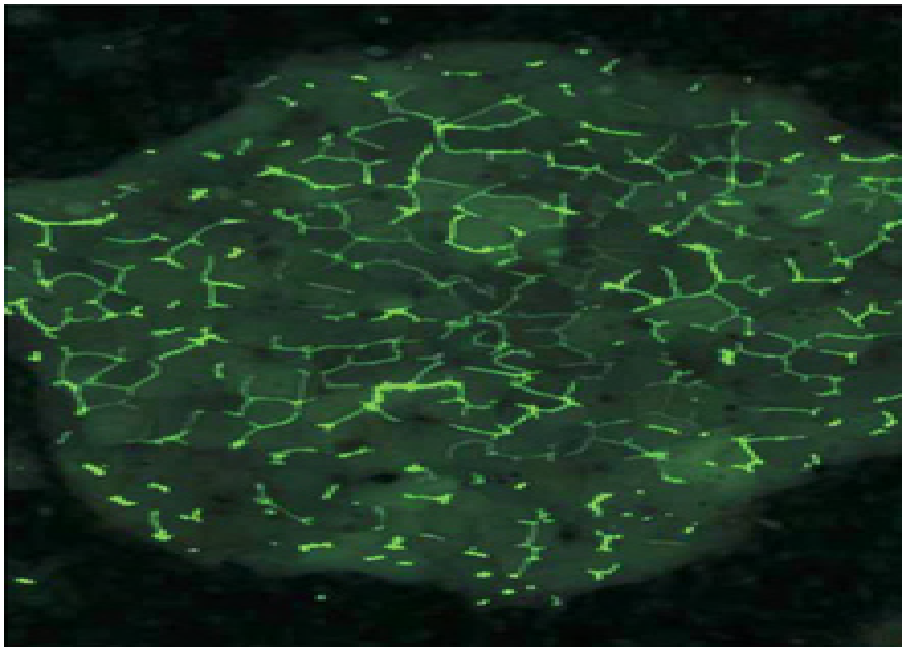
If you will, there’s a number of in-vitro and in silico models that are impressive.

In Vitro and *In Silico* Models

- SAR analyses
- Target tissue at risk
- Prediction of toxic metabolites
- Animal model microsome analysis
- Primary hepatocyte slices/cultures
- Promising new tools

  (eg microscale human hepatocyte cultures)

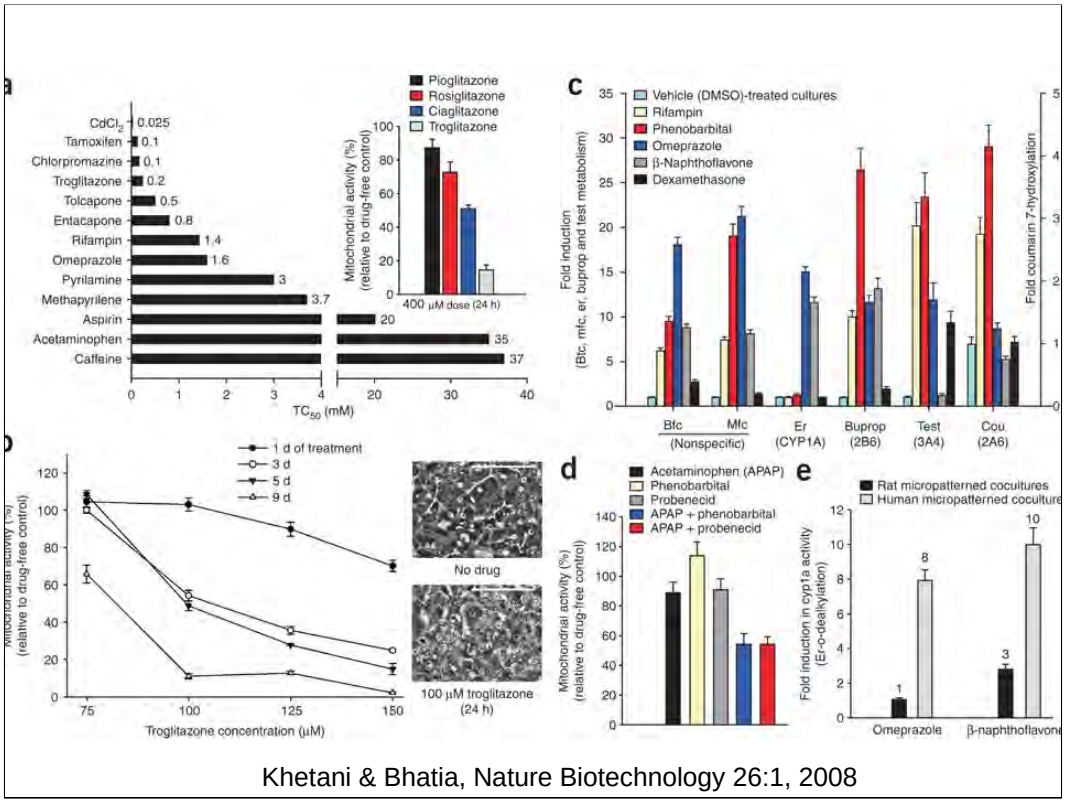
What is emerging can really in a fairly impressive way and the use of human tissues start to show SAR analyses, structure-activity relationship, target tissue at risk, prediction of toxic metabolites, animal model microsome analysis, et cetera, et cetera. And there are promising new tools, particularly the one I'm mentioning here, just to give you an example of the microscale human hepatocyte culture.



Khetani & Bhatia, Nature Biotechnology 26:1, 2008

This is a system that uses elastomeric stencils and semiconductor micro technology to create microscale hepatic tissue subunits with sustainable phenotypic functions. It's thought they have much higher function or demonstrate a higher level of function than suspension of individual cells or even liver slices. And, it's astonishing that enables the assessment of gene expression, Phase 1, 2 metabolism, canalicular transport, secretion of liver specific products and susceptibility to hepatotoxins. For example, in this particular photo micrograph, this is a group from MIT. This is probably Shirley, this year, very impressive it ends up to be reproducible and true.

Here's a slide of the micro pattern co-culture which is really demonstrating Phase 3 transporter activity, following incubation with dichlorofluorescein diacetate which is then internalized and cleaved by esterases and excreted into the bile cancaliculi by transporters and you can demonstrate this and measure it.



Khetani & Bhatia, Nature Biotechnology 26:1, 2008

This will be accessible to you in your materials and you obviously can't read all this but this shows you the power of a tool like this in screening for hepatotoxins

The first figure A here really represents the TC50 or toxic concentration that represents the 50 percent decrease in mitochondrial activity after 24 hours exposure, and the inset there is really the -- agents showing the differentiation as far as the impact of troglitazone. They go on to demonstrate the chronic toxicity in vitro by repeatedly dosing every 48 hours over a 2 week period showing the same kind of relative effect you could show as in the upper right, the effect of P450 induction with the appropriate drugs that do that over a three to four day period and then by adding the CYP450 substrates, you could show how you would have enhanced the backed up testosterone, bupropion, coumarin, et cetera.

You could take a drug interaction situation such as acetaminophen and showed you have increased toxicity to demonstrable with P450 induction with phenobarbital, glucuronidation as blocked by probenecid and this is really showing all three drugs versus the interaction and finally there's an example of species specificity as it relates to induction of CYP1A isoforms.

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
  -unprecedented targets and chemical entities
- New uses for established markers
- Genetic/molecular profiling

So this is impressive, some of these in vitro technologies that are coming before, but at issue is what it means. It depends on which patients you have to be able to apply this to and how relevant those patient populations are.

Mechanism-based screening, we had quite a few presentations relating to metabolic capability and toxic metabolites. We mentioned the P450 enzyme induction or inhibition, screening for genetic polymorphisms affecting metabolism and distribution, et cetera.

Metabolic Capability and Toxic Metabolites

- Association with induction/inhibition of P-450 enzymes.
- Screening for genetic polymorphisms affecting metabolism and distribution

  CYP and transporter variance
- Use of metabolomics
- Identification of toxic metabolites during preclinical and clinical development

I would point out specifically this screen for genetic polymorphisms, at Lilly, we've been collaborating with AffyMetrics, and what was part of Lilly, to really develop a chip that can simultaneously screen around 2,000 SNPs, specifically about 184 genes that are relevant to both Phase I and Phase II metabolism. They got over very significant technical hurdles in achieving that as it relates to the problems with PCR multiplexing where they used the same primer for all the amplicons, and then all the problems of hybridization causes resulting in interference, et cetera, et cetera.

Comprehensive Metabolic Enzyme/Transporter Analysis

Oxidation
Reduction
Hydrolysis

Conjugation:
Glucuronic acid **Amino acids**
Glutathione **Sulfuric acid**
Acetic acid
N-acetyl transferases
Glutathione-S transferases
Methyl transferases

Transporter Genes

Cytochrome P450 Genes

CYP2D6	**CYP2A6**
CYP2C8/9	**CYP2C19**
CYP1A2	**CYP3A4/5**
CYP2E1	**etc.**

Systematic Analysis

184 genes ~2,000 SNPs
Chip based platform under development

Two Sets of Genes:
Validated set – known clinical correlation
29 Genes – 171 SNPS
Additional genes – suspected clinical utility
155 Genes - ~1,800 SNPs

We are now screening all Phase I patients so that we have a record of this particular profile of the SNP450 and transporter enzymes so we will have this database to go back and access patients with that profile.

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
  -unprecedented targets and chemical entities
- New uses for established markers
- Genetic/molecular profiling

We've done broad Asian studies to demonstrate whether or not those populations are relevant as it relates to harmonization and whether some of the submissions are appropriate that are done outside the -- as it relates to Asian specific populations with drug metabolism, and we've had our first major submission that incorporates a lot of this pharmacogenomic data but again this is disproportionately skewed towards metabolism and to an emerging effect, hypersensitivity as we've been hearing.

Markers for Hypersensitivity Reactions

- Clinical signs, time course, lab features
- Autoantibodies to CYP

  eg halothane (CYP2E1), anticonvulsants (CYP3A4), dihydralazine (CYP1A2), tienilic acid (CYP2C9)
- Association with HLA genotype

  eg chlorpromazine, halothane, nitrofurantoin, clometracin, diclofenac, tricyclic antidepressants, ticlopidine, abacavir
- Assays for T-cell activation and cytokine release (TNF-α , IL1β, IL6, etc).

Speaking of hypersensitivity reactions, there are markers that we could use related to that. We heard about the clinical profile and laboratory profile that are important. Autoantibodies to CYP have been emerging as increasingly important as a mechanism and potentially biomarker showing susceptibility or characterizing what's happening.

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
  -unprecedented targets and chemical entities
- New uses for established markers
- Genetic/molecular profiling

Clinico-pathologic profiles,

Clinico–pathologic Profiles

Differential diagnosis for DILI

Specific lab tests, imaging and prodedures required to rule out:

- viral hepititis A, B, C
- autouimmune hepatitis
- shock liver
- biliary track disease
- alcoholic liver
- Malignancy
- other

I'm going to skip over these but obviously they're important for differentials

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
- New uses for established markers
- Genetic/molecular profiling

and we all understand that but

Clinico-pathologic “Signatures” as Biomarkers

- Most putative DILI agents show “signature effect”
- Well–characterized early signals can be useful in early detection of subsequent events
- Less helpful with unprecedented targets and new chemical entities
- Examples

  above risk factors

  clinical course

  patterns of enzyme elevations and other lab features

  pathological manifestations

they also provide the signatures that as we're moving forward, once we identify and try to characterize DILI, that we recognize these well-characterized early footprints or signals.

Examples of Pathological Manifestations that comprise a DILI Agent's "Signature"

- *Central necrosis*: acetaminophen, halothane, methoxyflurane, trovafloxacin, ketaconazole, dihydralazine
- *Hepatocellular degeneration/apoptosis with eosinophilic infiltrater +/- peripheral eosinophilia*: INH, pnenylbutazone, indomethacin, disulfiram
- *Lipofuscin pigment storage in hepatocytes*: phenothiazines, phenacetin, aminopyrine
- *Steatohepatitis*: aniodarone, nifedipine, didansine
- *Phospholipidosis*: perhexiline maleate, chloroquine, trimethoprim-sulfamethoxazole

Obviously that's helpful with unprecedented targets and new chemical entities but the combination of risk factors, clinical course, patterns of enzyme elevation and other lab features, and pathological manifestations which are associated with various kinds of putative DILI agents. So these are signatures that emerge.

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
  -unprecedented targets and chemical entities
- New uses for established markers
- Genetic/molecular profiling

New uses for established markers again,

New Uses for Established Markers

- Hy's Rule as a prototype
- Appropriate time course of liver testing
- Development and application of relevant reference data

  Lilly Reference Ranges

  Central Lab data as a resource

  unique opportunity?
- Analyses of intra-individual variation

  Use of Z value calculations

…and Hy's Rule is a great example of that, no longer new, certainly the appropriate time course for testing. We've heard a lot of presentations on that as far as the kinetics of enzyme elevations, et cetera, the development and application of relevant reference data. This is a very important one.

New Uses for Established Markers

- Hy's Rule as a prototype
- Appropriate time course of liver testing
- Development and application of relevant reference data
  - Lilly Reference Ranges
  - Central Lab data as a resource
- Analyses of intra-individual variation
  - Use of Z value calculations

At Lilly, many of you are aware that we developed a reference database based on 25,000 patients that we use in conjunction with other reference databases. For example, our central lab partner, Covance Central Laboratories, calculates that based on the central 99 percent and censor outliers. We use the central 98 percent without censoring outliers. It gives us a much broader range. It's broken down by demographics, drinking, smoking, et cetera, et cetera, et cetera.

ALT Reference Data
(U/Liter)

	NEJM Mass. Gen. 1992	CCLS Ctl 99% + censor	Lilly Ctl 98% no censor
Female	**7 – 30** (all ages)	**6 – 34** (< 70 yrs.)	**5 – 88** (< 50 yrs.)
		6 – 32 (> 69 yrs.)	**4 – 85** (> 49 yrs.)
Male	**10 – 55** (all ages)	**6 – 43** (< 70 yrs.)	**5 – 121** (< 50 yrs.)
Lilly Δ = +/- 27 U		**6 – 35** (> 69 yrs.)	**4 – 97** (> 49 yrs.)

Now this gives us the specificity we need. We get the sensitivity by establishing variation in specific sub-populations. So we have a statistic that you find data check (ph.) for ALT. For most of the populations, it happens to be around 27 units. And so that gives us the sensitivity we need to be used in, in conjunction with those broader reference ranges.

I should mention that the central lab database is hypothetically a huge resource that can be tapped with sponsor's consent. We have a poor understanding of predictive value. You heard some of the scary notions of sensitivity, specificity and incidence of the disease, defining predictive value and some of these profiles that we have for rheumatoid arthritis patients or patients with heart failure or renal failure, we need to get specific reference data in those populations and tapping into databases such as possible through central lab testing, which in essence is using common technical platforms that make the data combinable, does, does provide an interesting opportunity.

New Uses for Established Markers

- Hy's Rule as a prototype
- Appropriate time course of liver testing
- Development and application of relevant reference data

  Lilly Reference Ranges

  Central Lab data as a resource
- Analyses of intra-individual variation

  Use of Z value calculations

And finally, the use of Z value calculations

How do we define and identify important changes in lab values?

- as a value outside the normal range
- as a value outside a multiple of the normal range
- as a percent change from baseline
- as a percent change from baseline and greater than a fixed limit
- as a change from baseline (Z Value)

G Kapke Jan, 2008

that has been championed by Gordon Kapke at Covance which really

Total Result Variation (CV_T)

For a single laboratory value, the value lies within the range:

$$\pm Z * \%CV_T \text{ or } \pm Z * (\%CV_A^2 + \%CV_I^2)^{0.5}$$

with the probability associated with the Z value.

G Kapke, Jan, 2008

uses a combination of individual variation and analytical variation to calculate the individual variation to be using and interpreting individual patient data. So our Lilly data check slogan at individual population variation where this looks at individual patient variation.

Categories of Biomarkers Applicable to Detection and Management of Hepatotoxicty (DILI ?)

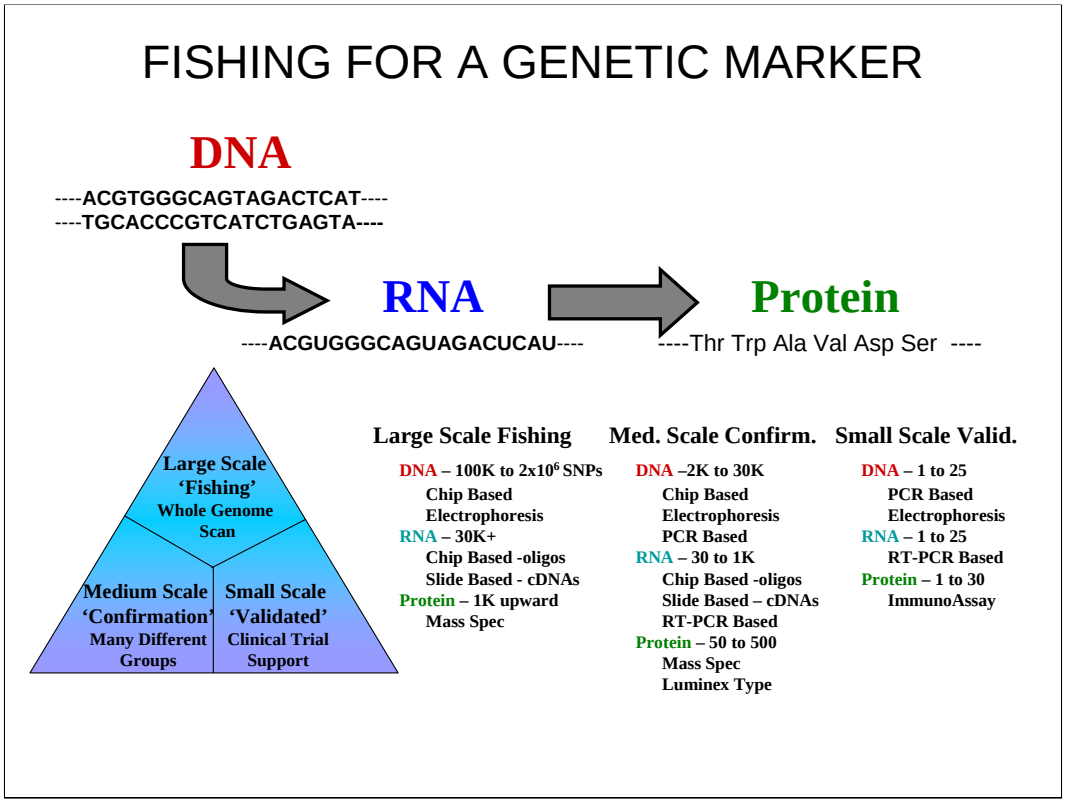
- Established risk factors
- *In-vitro* and *in silico* models
- Mechanism–based screening
  metabolic capability and toxic metabolites
  hypersensitivity reactions
- Clinico–pathological profiles
  differential diagnoses
  agent/class–specific “signatures”
  -unprecedented targets and chemical entities
- New uses for established markers
- Genetic/molecular profiling

And then finally, the genetic/molecular profiling.

Genetic/Molecular Profiles Associated with Patients at Risk

- Impact of available technologies
  DNA vs. other molecular analyses
  eg pyrosequencing
- Relevant patient populations
- The numbers game: finding associations with rare ADEs
- Access to appropriate annotated specimens
- Examples of agent-specific correlations
- Rationale for screening aggregate pools at DILI patients.
- “Relative high incidence” idiosyncratic toxicity detectable premarketing– the rare opportunity
  eg. Abacavir: 5-8% HLA-B*5701 allele

We have the impact of available technologies. Why are we looking disproportionately at DNA? It's because of that odd colored comment about why dogs do certain unattractive things. It's because we can. We have things like pyrosequencing, the ability to do high volume sequencing of literally millions of variances, many genes across multiple individuals and with partners like -- who are able to generate an enormous amount of information which we really cannot do on proteins, RNAs and other such things. So the technology is defining the possible.



Again, we have the importance of relevant patient populations which I've mentioned. We have the numbers game of finding associations with rare adverse events. This slide simply makes the point of how we start fishing broadly, looking at DNA through whole genome scans and then as we increasingly narrow down the, the possible targets, we're able to employ different technologies to do that before we can get the serviceable biomarker.

Genetic Variance (SNPs/ polymorphisms) as Biomarkers

- Disease Susceptibility Biomarkers
  - Single Disease Genes (Mendelian Inheritance)
  - Genetic Associations in Complex Diseases
  - Genetic Changes Associated with Tumorogenesis
- Drug Activity Biomarkers
  - Genetic Polymorphisms Predicting Drug Metabolism
  - DNA Variations Predicting Drug Response
  - DNA Variations Predicting Adverse Events

With genetic variance or SNPs, as biomarkers, we're talking about disease susceptibility biomarkers and drug activity biomarkers and here we're interested in mainly genetic polymorphisms predicting drug metabolism and DNA variations predicting adverse events.

Association Study: Keys to Success

Characteristics of the Putative Allele

Sample Size Considerations

Relative Risk	1.5	**2.0**	2.0	2.0	2.0
Allele Frequency	.5	.5	.5	**.1**	.1
Alpha	.05	.05	.05	.05	**.001**
N for each group	600	165	235	125/1125	260/2340
Total Sample	**1200**	**330**	**470**	**1250**	**2600**

Most disease susceptibility polymorphisms show a relative risk of approx 1.5-1.8. To have clinical utility as a predictive marker for an AE, a relative risk of 5-10 is required.

Definitions

Relative risk: relative risk associated with carrying the putative SNP
Allele frequency: the frequency of the SNP associated with response
Alpha: Type I error rate

But the numbers, particular in the latter, are very much against us because how we establish the relative risks and other components, that really determine whether or not these associations present a serviceable biomarker is really quite daunting.

I make the point there that most disease susceptibility polymorphisms show a relative risk of approximate 1.5 to 1.8, and to have a utility as a predictive marker for an adverse event, a relative risk of 5 to 10 is required.

Table 1. Pharmacogenomic Biomarkers as Predictors of Adverse Drug Reactions. NOTE INCIDENCE

Gene or Allele	Relevant Drug	Specificity of Biomarker	Percent of Patients with an Adverse Reaction to Drug*
TPMT (mutant)	6-Mercaptopurines	Very good	1–10
UGT1A1*28	Irinotecan	Good	30–40
CYP2C9 and *VKORC1*	Warfarin†	Good	5–40
CYP2D6 (mutant)	Tricyclic antidepressants	Relatively good	5–7
HLA-B*5701	Abacavir	Very good	5–8
HLA-B*1502	Carbamazepine	Very good	10
HLA-DRB1*07 and DQA1*02	Ximelagatran	Good	5–7

* Percentages are of affected whites except that for HLA-B*1502, which is the percentage of affected Asians.
† Carriage of the *CYP2C9* and *VKORC1* alleles affects warfarin dosing.

M Ingelman-Sundberg, N Engl J Med 2008; 358:637

Now granted, that's going to be done within a larger context of information and I'm not downplaying the usefulness of such associations but as far as finding magic markers, that ain't going to get us here.

The -- information that came out earlier this month as was published relating to abacavir and the accompanying editorial in the New England Journal, gave a table that summarized pharmacogenomic biomarkers as predictors of adverse reactions, and I just call your attention the incidence or the percent of patients with these adverse events. They're all high enough to allow you to make those explorations, arguably even premarketing.

Other Challenges in Finding Genetic Biomarkers for Rare AEs

- Study design requirements

  well-defined case and control groups
- Reliance on retrospective and nonblind study protocols
- Suboptimal selection of gene variants

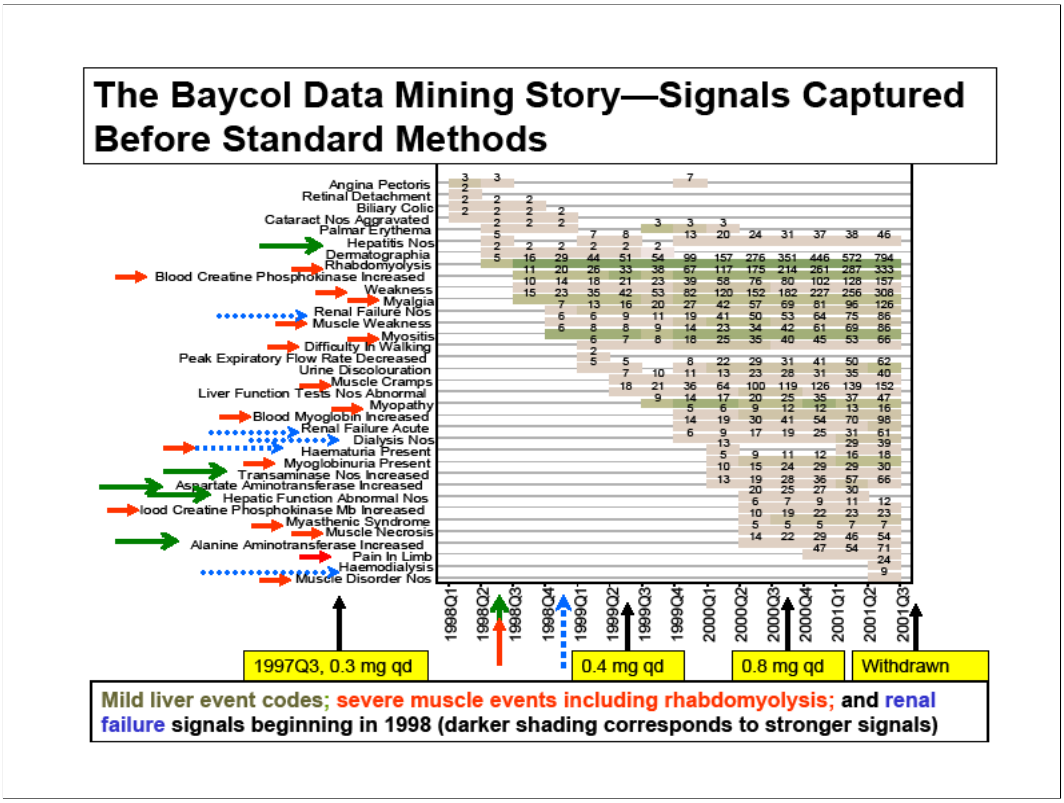
  eg challenges in linking gene expression changes with reactive metabolites
- The likelihood of polygenic influences
- Concomitant medications

Other challenges, study design requirements, well-defined case and control group, reliance on retrospective and nonblind study protocols, suboptimal selection of gene variants. This has been particularly challenging with looking at linking gene expression to changes in reactive metabolites. The likelihood of polygenic influences, that's very important when we look at the numbers game, and then, of course, concomitant meds.

Challenges in Using Pre-marketing Clinical Trial Subjects Showing DILI Signals to Find New Biomarkers

- Accessibility of candidate DILI patients (Rule of Three)
  KEY ISSUE: relevance of more common hepatotoxicity
- Detecting relatively high incidence DILI (eg,isoniazid)
- Clinical trials not routinely powered or designed to detect and characterize low incidence safety signals
- Ability to define causality
- Access to adequate number of annotated specimens
- Lack of standardized data collection and storage

So we have a series of challenges that relate to the premarketing clinical trial subjects and how we detect DILI signals to find new biomarkers. The accessibility of the candidate DILI patients, the Rule of Three. We talked through that. Detecting relatively high incidence DILI, cases like Isoniazid notwithstanding. Clinical trials are not routinely powered or designed to detect and characterize low incidence safety signals. We've all talked through that extensively. The ability to define causality, access to adequate number of annotated specimens and lack of standardized data collection and storage.



I'm going to skip over the Baycol example, which is a wonderful example of how you had both in terms of preclinical, pre-registration, ongoing development and post-marketing, data that was not able to be brought to get literature really to detect in a timely fashion, the renal failure that I hope you will be able to do in a better situation today. We go back to the time permitting.

Challenges in Using Post-marketing DILI Candidate Patients to Find New Biomarkers

- Inadequacies associated with voluntary reporting
- Need for timely identification of well-characterized candidate (high probability) DILI patients

  Progress in post marketing signal detection
- Access to biologic specimens and related data required for prospective and retrospective biomarker research
- Need for standardized, accessible electronic medical records

  Emerging data standards (CDISC, HL7, ONCHIT and other HHS initiatives, industry-gov working groups)

  Use of emerging networks (HMO/insurers/payors, hospital networks, CDC, NCI, other)
- **Determining causality**

So there're also challenges in using post-marketing DILI candidate patients to find new biomarkers. There's the inadequacy associated with voluntary reporting. I know John and others think that's a show stopper and to some extent I agree along with the need for timely identification of well-characterized high probability candidate DILI patients, progress in post-marketing signal detection notwithstanding. Access to biologic specimens and related data required for prospective and retrospective biomarker research, we've mentioned that. The need for standardized, accessible electronic medical records, and we do have emerging standards that are promising as far as getting over that particular obstacle as well as some of the emerging networks in terms of the various HMOs, CDC, as well as the DILI Network, the Pharmacogenomics Research Network, et cetera, et cetera.

Challenges in Using Post-marketing DILI Candidate Patients to Find New Biomarkers

Determining causality: *familiar challenges*

- Nature of rare serious (idiosyncratic) ADE
- High incidence “background” ADE
- Concomitant meds/ little drug-drug interaction data
- Unexpected vs pre-specified harm
- “Signal” inadequately defined
- Use of markers with low or poorly defined predictive value
- Trend toward sophisticated interventions with unprecedented targets

Determining causality is still a problem and I won't rehash what has already been gone through as far as the causality problem.

Summary and Conclusions

The discovery and validation of biomarkers for an idiosynchratic toxicity like DILI requires timely access to a statistically-defined number of well-characterized affected patients, appropriate case controls and adequately annotated biologic specimens. Challenges in achieving this postmarketing have included unreliable reporting, inadequate or inconsistent characterization, concomitant treatments and comorbidities, and other circumstances that complicate the determination of causality and access to the data and tools required to identify and validate a serviceable biomarker. While there are opportunities to overcome some of these barriers in the well-controlled pre-marketing clinical development environment, access to enough affected patients provides an even greater hurdle. Issues that could mitigate these restrictions include the relevance of “relatively high incidence” DILI and other (dose-dependant) hepatotoxicity, and whether patients with DILI associated with different agents share common predisposing biologic characteristics. Accordingly, the prospect of finding new biomarkers for DILI would be enhanced by combining and coordinating our pre- and postmarketing research efforts and resources.

And with that, I'll summarize with this rather wordy paragraph which emphasizes that the discovery and validation of markers for an idiosyncratic reaction like DILI requires timely access to a statistically-defined number of well-characterized affected patients, appropriate case controls and adequately annotated biologic specimens. Challenges in achieving this post-marketing have included unreliable reporting, inadequate or inconsistent characterization, concomitant treatments and comorbidities and other circumstances that complicate the determination of causality and access to the data and tools required to identify and validate a serviceable biomarker.

So while there are opportunities to overcome some of these barriers in the well-controlled pre-marketing clinical development environment, access to enough affected patients provides an even greater hurdle obviously and that's what we can be debating following this session. Issues that could mitigate these restrictions include the relevance of relatively high incidence DILI and other dose-dependent hepatotoxicity, and whether patients with DILI associated with different agents share common predisposing biologic characteristics.

So accordingly, the prospect of finding new biomarkers for DILI would be enhanced by combining and coordinating our pre and post-marketing research efforts and resources.

ACKNOWLEDGEMENTS

Richard Hockett, MD. Lilly
Arie Regev, MD, Lilly
Ana Szarfman, MD, PhD, FDA

And with that, I'll stop.