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Daiichi-Sankyo

2014 JSSX Award for Young Industrial Scientists

Risk Assessment of the Chemically Reactive Metabolites and Idiosyncratic Drug Toxicity in Drug Development

29th JSSX Meeting

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Contents

- Zone classification system for the risk assessment of idiosyncratic drug toxicity
- Risk assessment strategy to mitigate the formation of RMs in the early discovery stage
- Identification of the cellular response to the formation of covalent binding

Contents

- **Zone classification system for the risk assessment of idiosyncratic drug toxicity**
- **Risk assessment strategy to mitigate the formation of RMs in the early discovery stage**
- **Identification of the cellar response to the formation of covalent binding**

Understanding IDT

- IDT causes life-threatening toxic events (even death) in patients
- IDT can not be easily predicted from non-clinical toxicity studies
- Often only appears in post-marketing with low frequency (1/100 - 1/100,000)
- Leads to “Black Box Warnings”, or drug withdrawal.
  - Ticlopidine (Hepatotoxicity, TPP)
  - Troglitazone (Hepatotoxicity)
  - Tienilic acid (Hepatotoxicity)
  - Aminopyrine (Agranulocytosis)
- Mechanisms of IDT are not fully understood

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Mechanism is Unclear but…

Reactive metabolite and its covalent binding to cellular macromolecules

- Many drugs associated with IDT are known to form reactive metabolites to bind to proteins covalently

Exposure of the drug

- The occurrence of IDT is rare with drugs given at a daily dose of 10 mg or less

Host –specific genetic, environment, and/or disease factors

- IDT does not occur in most patients

Objective

In order to clarify the correlation between amount of CB, daily dosage and risk of IDT, we performed the covalent binding study using 42 drugs with several safety profiles

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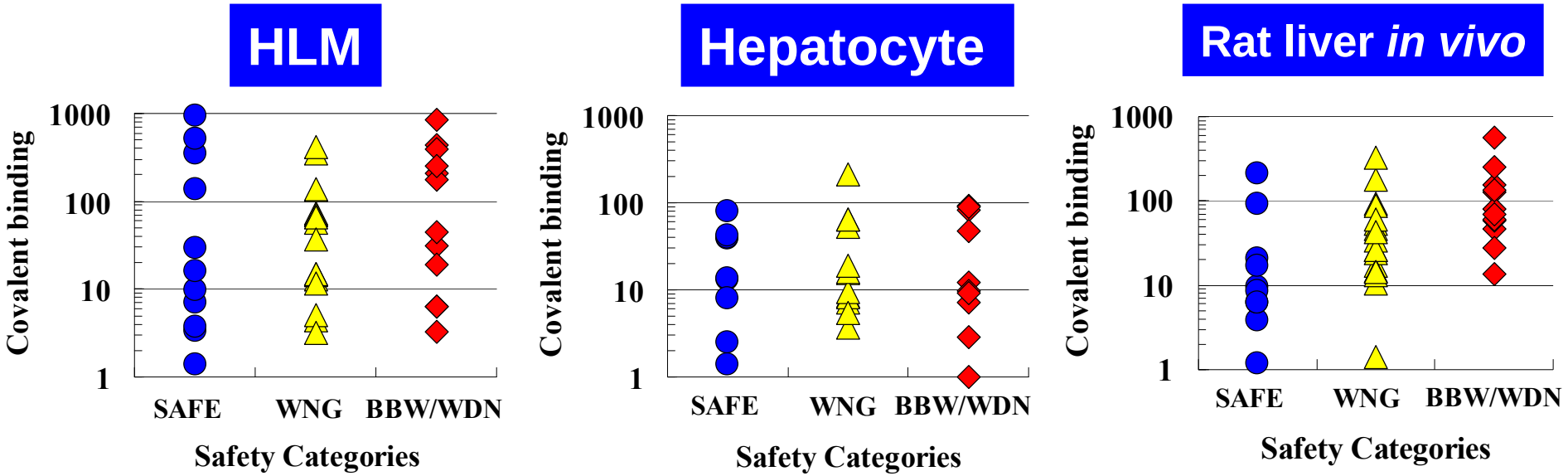
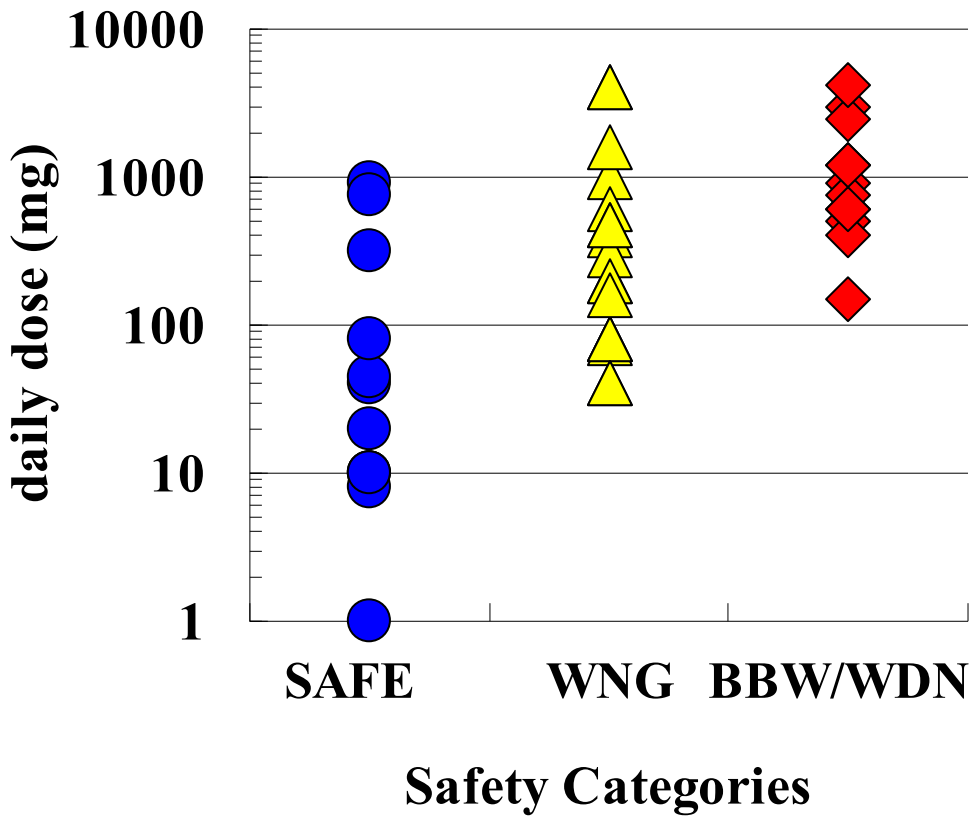
Covalent Binding Categorized by the Safety Profile



- C.B. in three systems could not distinguish the safety categories

Nakayama et. al., Drug Metab Dispos. (2009)

Daily Dose Categorized by the Safety Profile



Daily dose could not distinguish the safety categories clearly

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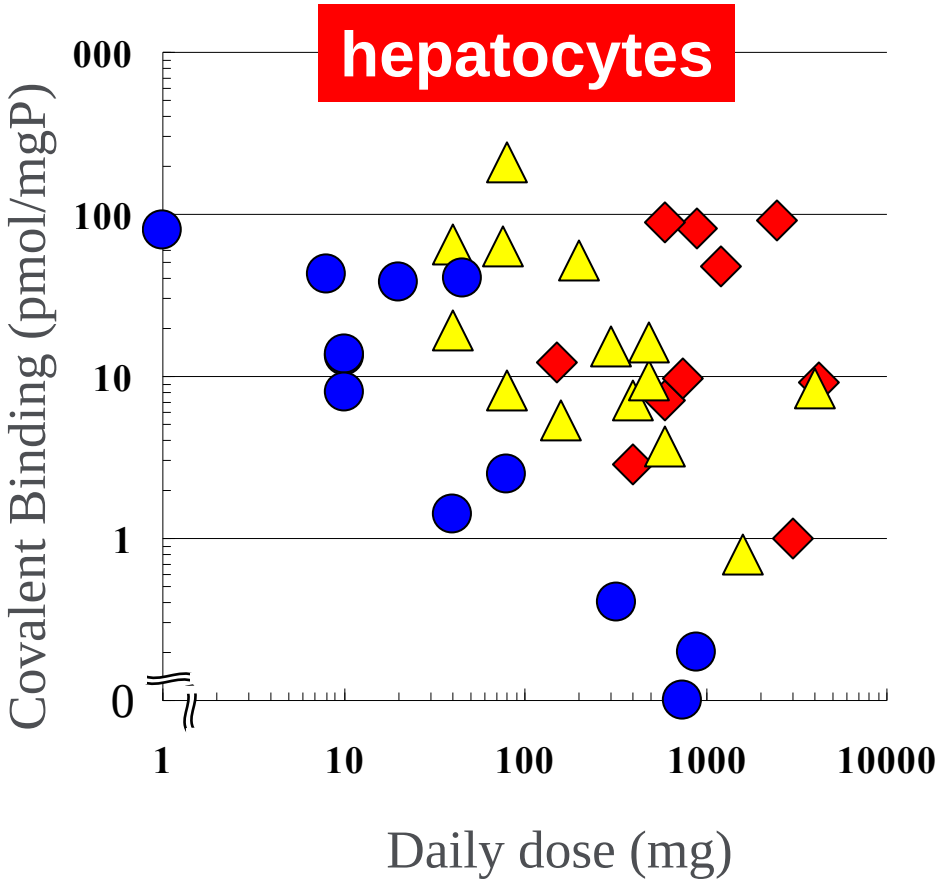
Correlation of Daily Dose and Covalent Binding Categorized by the Safety Profile



Both covalent binding and daily dose are related to the occurrence of IDT independently.

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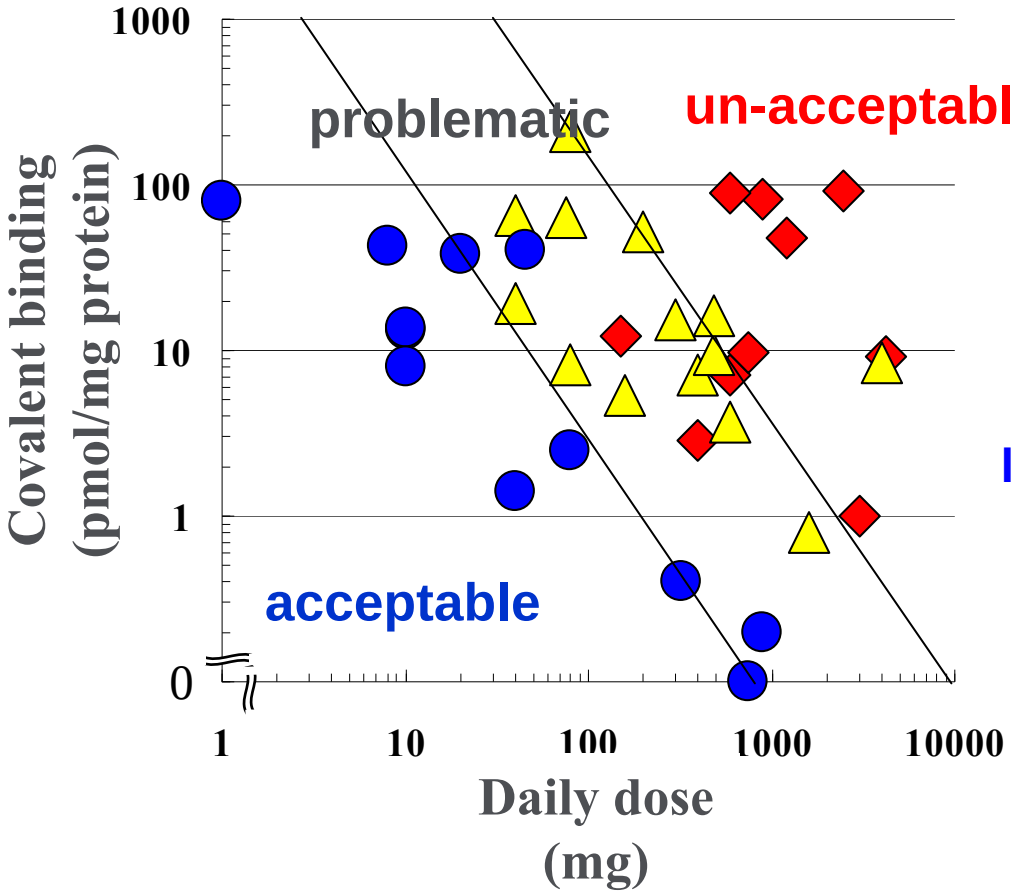
Zone Classification System

Categorization of Safety Profile by the amount of Covalent binding and daily dosage



From the results of ordinal logistic regression analysis

Logit: log(p/1-p)

p = 0.5

$$\log(CB) = \beta_0/\beta_2 - \beta_0/\beta_1 \times \log(dose)$$

- **Almost all drugs were located in zones corresponding to their respective classified safety categories.**

Nakayama et. Al., Drug Metab Dispos. (2009)

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Screening method for the detection of RMs

Trapping Assay

○: High throughput, structural elucidation

×: Can not detect all RMs

Time dependent CYP inhibition Assay

○:High throughput　DDI risk assessment

×: Can not detect all RMs

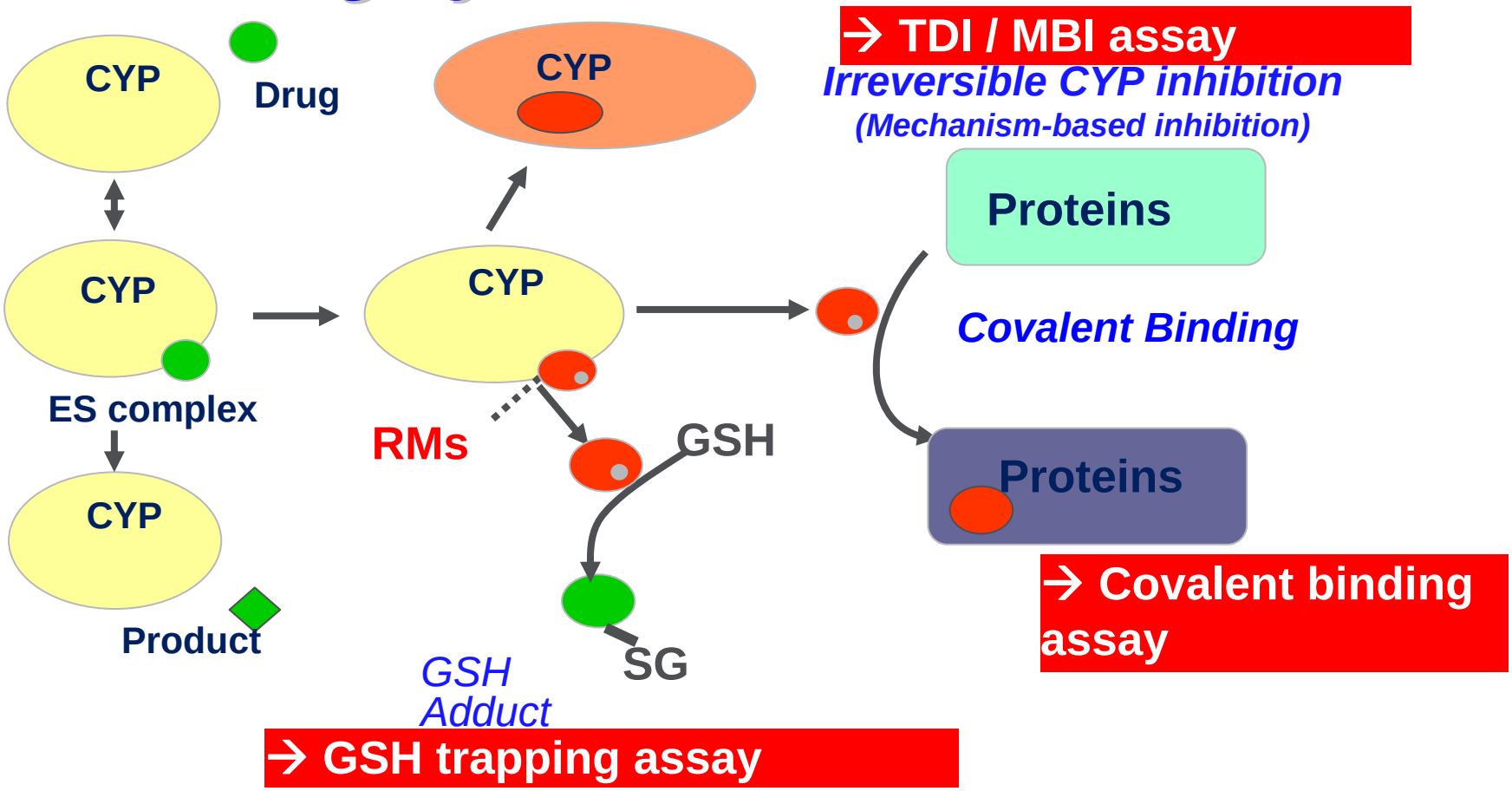
Covalent Binding Assay

○: Directly detected to the binding to macromolecule

×: Low throughput, necessity of labeled compounds

Qualitative trapping assay using agents such as glutathione (GSH) is often performed in the early stages of drug discovery

Fate of the Reactive Metabolites and Detecting System

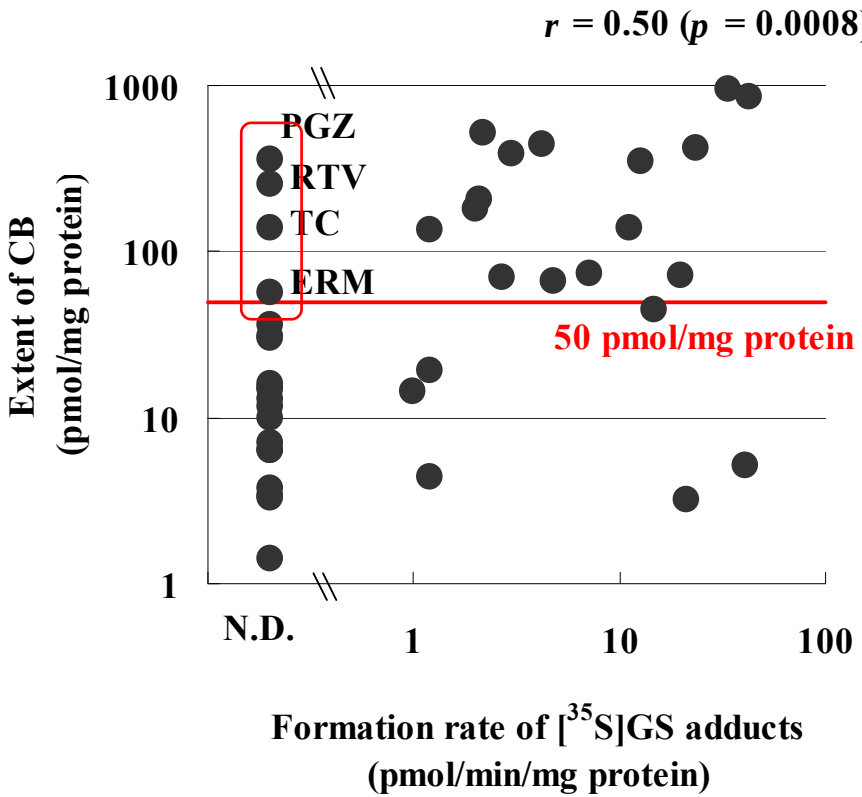


We hypothesized that TDI assays could be complementary to trapping methods.

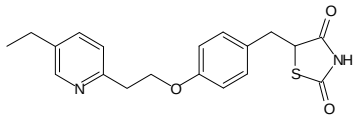
Objective

In order to clarify the importance of TDI assays in detection of RMs, we investigated the relationships between the formation rate of [^{35}S]GS adducts, the enzyme inactivation rate in the TDI assay, and the extent of CB in HLMs

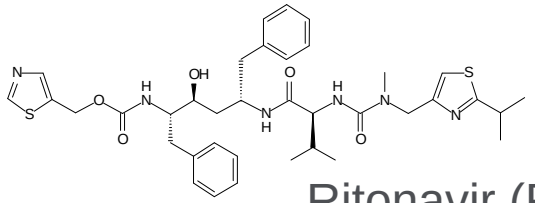
Correlation between [^{35}S] GSH Adduct and Amount of Covalent Binding



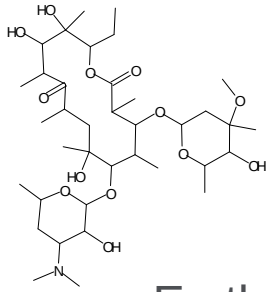
r is the Spearman rank correlation coefficient.



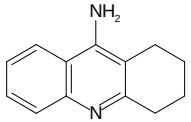
Pioglitazone (PGZ)



Ritonavir (RTV)



Erythromycin (ERM)



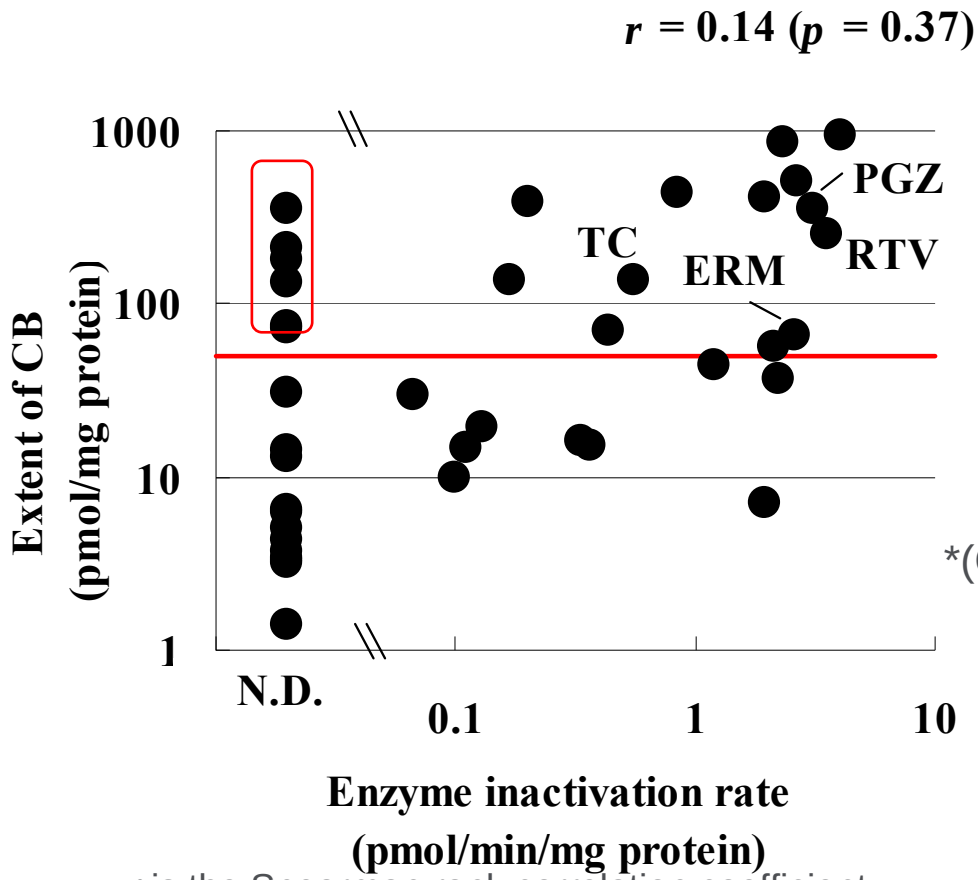
Tacrine (TC)

Four compounds with high level of CB did not show detectable GS adduct peaks

→the trapping methods alone cannot replace the CB assay

Nakayama et. al., Drug Metab Dispos. (2011)

Correlation between Enzyme Inactivation rate and Amount of Covalent Binding



r is the Spearman rank correlation coefficient.

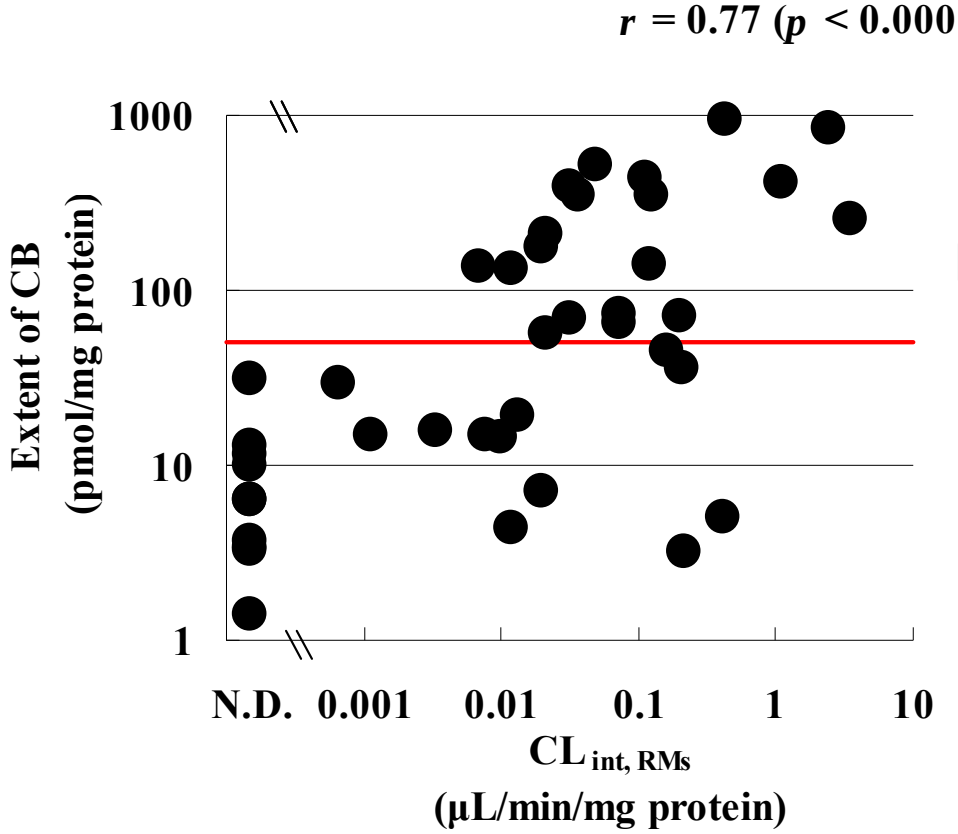
Enzyme Inactivation rate=

$$\Sigma * \left(\frac{[\text{inactivation\%}] \times [\text{CYP contents}]}{[\text{pre-incubation time}]} \right)$$

*(CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4)

Four compounds that the GSH trapping assay failed to detect were inactivators of the enzyme in the TDI assay

Correlation between RMs intrinsic clearance and covalent binding



r is the Spearman rank correlation coefficient.

To combine parameters from the two assays

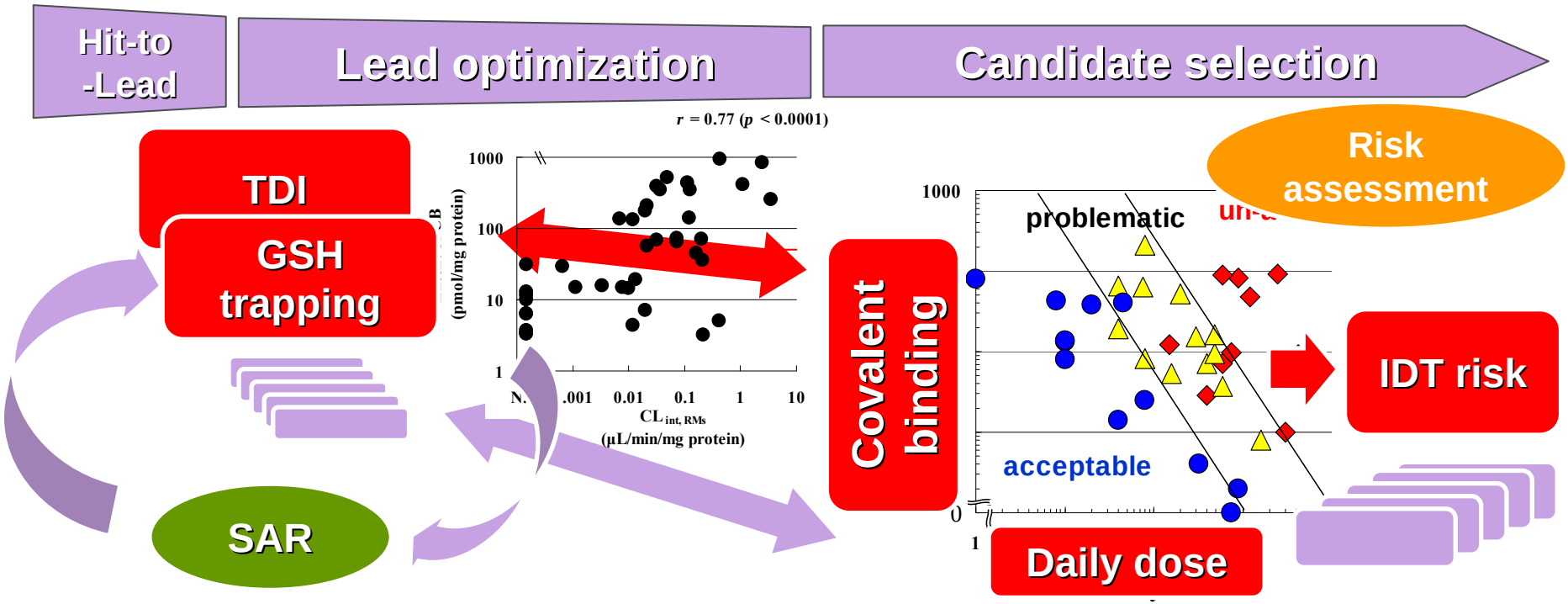
RMs intrinsic clearance ($CL_{int.RMs}$):

$$= \frac{\text{Formation rate of } [^{35}S] \text{ GSH adduct}}{\text{Substrate Conc.}} + \frac{\text{Enzyme inactivation rate}}{\text{Substrate Conc.}}$$

Correlation of Clint, RMs to CB was better than that of GS adduct

A combination of the GSH trapping and TDI assays is an effective method for detecting compounds with the potential of highly reactive metabolites

Risk assessment strategy for reactive metabolites in drug discovery



Risk lowering phase

Detailed assessment phase

Screening by GSH trapping assay and TDI assay

Risk assessment by a Zone classification system

Contents

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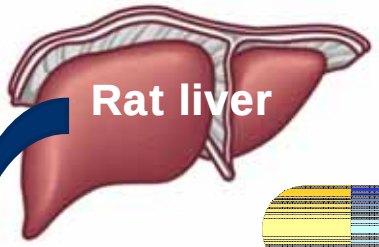
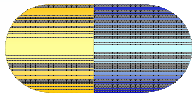
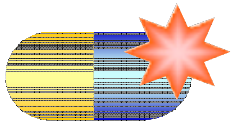
Introduction

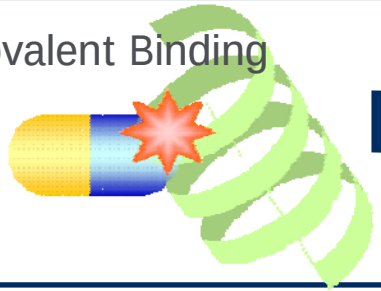


Reactive metabolites

Covalent Binding







TG-GATEs

トキシコゲノミクスプロジェクト（TGP）

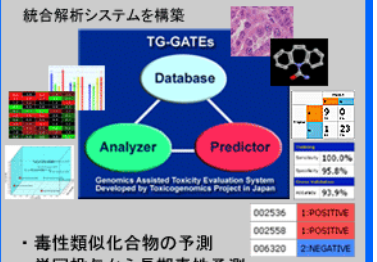
期間：2002 ～ 2006
官民共同研究

医薬基盤研究所，国立医薬品食品衛生研究所，製薬企業 15 社

創薬初期段階の効率化
⇒ 参加企業で運用開始

150 化合物の毒性データ・遺伝子発現データを取得
品質・データ量で世界最大規模

TG-GATEs を開発
Database（データベース），Analyzer（解析），Predictor（安全性予測）からなるトキシコゲノミクス統合解析システムを構築



・毒性類似化合物の予測
・単回投与から長期毒性予測
・医薬品候補化合物のランク付け
・毒性メカニズム解析

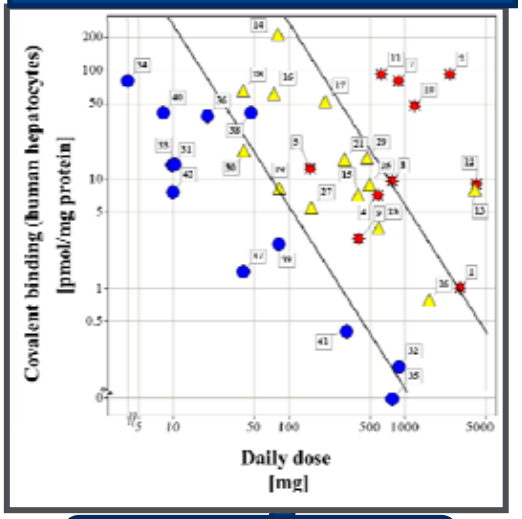
http://wwwtgp.nibio.go.jp/

Tox & gene expression profile

Black Box

IDT

Covalent binding study



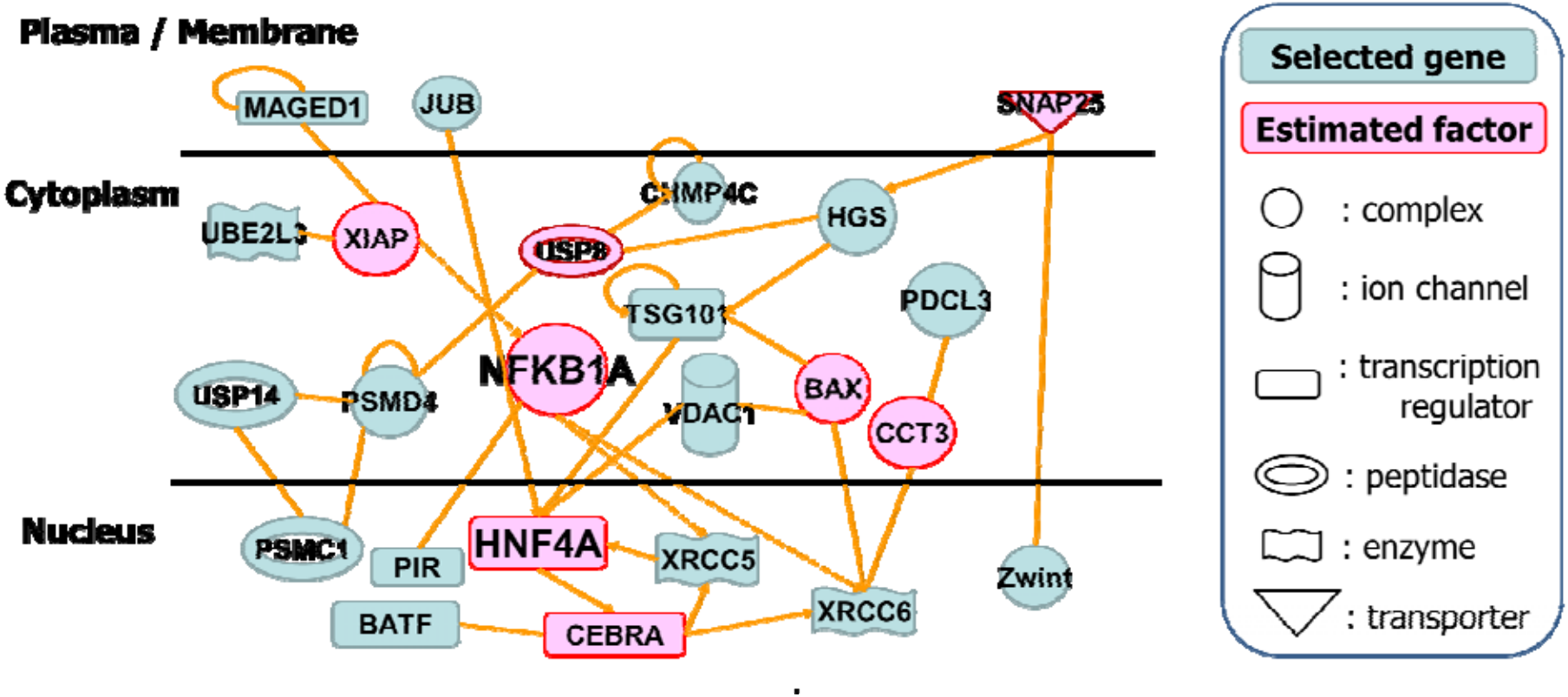
CB & daily dosage

Selected Gene Probe Sets Categorized by the Function

Affimetrix probe set ID	Correlation coefficient	Gene symbol	Gene title
DNA repair			
1370931_at	0.60	Xrcc5	X-ray repair complementing defective repair in Chinese hamster cells 5
1370537_at	0.55	Xrcc6	X-ray repair complementing defective repair in Chinese hamster cells 6
Ubiquitin / proteasome			
1371944_at	0.65	Ube2l3	ubiquitin-conjugating enzyme E2L 3
1398792_at	0.58	Psmc1	proteasome (prosome, macropain) 26S subunit, ATPase, 1
1386930_at	0.57	Psmd4	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4
1383073_at	0.55	Usp14	ubiquitin specific peptidase 14
1368817_at	0.51	Psme4	proteasome (prosome, macropain) activator subunit 4
Apoptosis			
1373052_at	0.59	Pdcl3	phosducin-like 3
1386909_a_at	0.58	Vdac1	voltage-dependent anion channel 1
Cell proliferation			
1386895_at	0.66	Maged1	melanoma antigen, family D, 1
1373122_at	0.64	Jub	jub, ajuba homolog (Xenopus laevis)
1370803_at	0.58	Zwint	ZW10 interactor
Epigenetics, gene transcription and signal transduction			
1382663_at	0.57	Batf	basic leucine zipper transcription factor, ATF-like
1390944_at	0.56	Chmp4c	chromatin modifying protein 4C
1372122_at	0.54	Tsg101	tumor susceptibility gene 101
1367840_at	0.53	Hgs	hepatocyte growth factor-regulated tyrosine kinase substrate
Oxidative stress / cell proliferation			
1377662_at	0.62	Pir	pirin (iron-binding nuclear protein)
Oxidative stress (excluded from gene set)			
1372523_at	0.56	Gclc	glutamate-cysteine ligase, catalytic subunit
1369061_at	0.56	Gsr	glutathione reductase
1368037_at	0.55	Cbr1	carbonyl reductase 1 /// inducible carbonyl reductase-like
1370688_at	0.54	Gclc	glutamate-cysteine ligase, catalytic subunit
1388122_at	0.52	Gstp1	glutathione S-transferase pi 1
1398753_at	0.51	Akr1a1	aldo-keto reductase family 1, member A1 (aldehyde reductase)

A total of 65 genes showed statistically significant correlation between the expression level and CB level, and 16 genes were selected as CB-associated genes based on their biological functions

Ingenuity Pathway Analysis of Genes Related to Formation of CB



Following the CB formation….

- **Adaptive response was occurred**
- **HNF4a and NFkB would be candidate key factors**

Summary

- **We established a zone classification system using covalent binding in human hepatocytes and a daily dose for the risk assessment of IDT.**

- **We demonstrated that a combination of the [^{35}S]GSH trapping and TDI assays is an effective method for detecting compounds potentially capable of generating highly reactive metabolites in the early stages of drug discovery.**

- **We estimated the molecular response against RM and CB formation by correlation analysis between the formation of the CB and TGx data.**

Acknowledgment

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