

S-4

## Toxicogenomics Data Generation for Predictive Biomarker Discovery

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### EDUCATION

- |      |  |
|------|--|
| 1995 | Kangwon National University, Korea<br>Veterinary Immunology M.S. |
| 2000 | Hokkaido University, Japan, Toxicology<br>Toxicology Ph.D.       |
| 1992 | Kangwon National University, Korea Veterinary Medicine<br>D.V.M  |
| 2005 | Diplomate of the Korean Board of Toxicology<br>DKBT              |

### EXPERIENCE

- |              |   |
|--------------|---|
| 2000–2002    | Post-doctoral fellow, Karolinska Institutet, Sweden   |
| 2002–2003    | Research Professor, Hanyang University, Korea   |
| 2003–present | Senior Research Scientist, Korea Institute of Toxicology, Korea   |
| 2004–present | Adjunct Assistant professor: University of Science & Technology(UST)<br>-Biopotency/Toxicology Evaluation |
| 2004–present | Team Leader, Toxicogenomics Team, KIT   |

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for Predictive Biomarker Discovery

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Korea Institute of Toxicology

## Contents

1. Toxicogenomics
2. Biomarker
3. Data generation
4. Perspectives

### 1. What is Toxicogenomics?

- TG is a young science- 1993, Pat Brown (Stanford Univ.)
- TG does not replace "Classical Toxicology"
- TG can be used in pre-clinical drug development to assess the safety of candidate drug
- TG has the potential to be a useful tool for regulatory decision making...

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### The other side of TG

- Understand the benefits and shortfalls of the technology
- Know how to evaluate and interpret the data
- Have a mechanism that allows sponsors to share this (exploratory) data with us (on a voluntary basis)

### Why Toxicogenomics?

- Better understanding the mechanism of toxicity
- Predict/detect toxicity earlier
- Assess pre-clinical toxicity at the molecular level
- Make better recommendations for pre-clinical safety studies
- Learn about validating genomic biomarker
- Learn about the application & use of new technique

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### Current Issues

- TG biomarkers need to be validated :
  - Toxicity: to avoid excluding potentially good drug candidates
  - Safety: to confirm that the absence of a signal corresponds to a safe compound

## Key questions

- ⦿ Which toxic compounds should be tested
- ⦿ Which controls should be used
- ⦿ How many toxic and control compounds should be included
- ⦿ Which dose (range) should be tested
- ⦿ Which time points should be chosen
- ⦿ How many replicated are needed
- ⦿ Which genes should be included

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## 2. Biomarkers in Toxicogenomics

- ⦿ DMPK: transcriptional regulation of P450s, drug transporters, 2ndary effects on metabolism, endocrine,etc
- ⦿ Pharmacology: known, expected, or biologically compelling efficacy markers
- ⦿ Correlation with NOEL: global expression profile as a marker of significant histopathologic or other toxic effect
- ⦿ Mechanism of Toxicity: transcription in target vs. non-target tissues, elucidation of time & dose response, characterization of transcription relative to tool positive/negative control compounds, pathways, pathology score, etc.

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## Classification of Biomarkers

- ⦿ **Known valid**
    - accepted by scientific community at -large to predict clinical outcome
  - ⦿ **Probable valid**
    - appears to have predictive value but not yet replicated or widely accepted
- \* Classification leads to specifications for validation in the context of intended use for biomarker

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## Classification of Biomarkers

- ⦿ **Exploratory Biomarkers**
  - lay groundwork for probable or known valid biomarkers
  - :Hypothesis generation
  - Fill in gaps of uncertainty about disease targets, variability in drug response, animal-human bridges and new molecule selection : Learn and improve success in future drug development programs
  - Can be "de novo" or "sidebar" study embedded in (pivotal) clinical efficacy trials

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## Known valid biomarkers

- ⦿ **Example from drugs**
- Safety:
  - \* TPMT(6-MP, Azathioprine)
  - \* UGT1A1(Irinotecan)
  - \* CYP2C9/VKORC1(Warfarin)
  - \* CYP2D6(Strattera)
- Efficacy:
  - \* EGFR status (Erbitux, Tarceva)
  - \* Her2/neu status(Herceptin)
  - \* Philadelphia chromosome ~ Bcr-abl(Gleevec)
  - \* C-kit(Gleevec)

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## Probable valid biomarkers

- ⦿ **Example**
- Safety:
  - \* Kim1 ~ preclinical (nephrotoxicity)
  - \* Gene panels used for preclinical safety evaluation
- Efficacy:
  - \* EGFR mutations (Iressa)
  - \* CYP2D6(Tamoxifen)
  - \* OncotypeDx gene panel( radiation therapy)

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## Exploratory biomarkers

- Examples

- Safety:  
\*Gene panels used for preclinical safety evaluation
- Efficacy:  
\*APOE4(Donepezil, Alzheimers)  
\*VEGF(several anticancer agents)  
\*Adiponectin mutations(rosiglitazone, type 2 diabetes)

*[Modified from US FDA documents]*

*[KIT > Biomarkers in  
Biomarker Development]*

## Biomarkers in Drug Discovery & Development

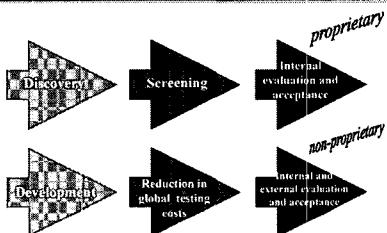


Fig. 1. Goals for biomarkers in pharmaceutical discovery and preclinical and clinical development.

*[Quoted from J. Pharm & Tox. Methods, Ghodmid, 49:183-186, 2004]*

*[KIT > Biomarkers in  
Biomarker Development]*

## How to make validated biomarkers?

- Most known valid biomarkers have been "validated" by accumulating data over many years
- Markers for "targeted therapies" become known valid when treatment is approved: they are used to demonstrate efficacy during clinical drug development
- FDA pharmacogenomics guidance does not provide information about marker validation
- Short of clinical trials in drug development process, there are no established processes of marker validation or are prospective studies required?
- A validation path for pre-clinical markers has been proposed

*[Modified from US FDA documents]*

*[KIT > Biomarkers in  
Biomarker Development]*

## Guidelines

### Pharmaceuticals

*[Topic Area: Metabolism/Exposure]*

#### Type of Submissions

Required in full report (IND)

#### Reasons

The sponsor uses the test results to "support scientific arguments pertaining to the pharmacological/metabolic mechanism of action, the kinetics of drug delivery or the safety and effectiveness of a drug" (as described in Figure A-2 of this document).

**Strategic 1:** During IND development, a sponsor conducts single- and multiple-dose pharmacokinetic studies via a more molecular safety (MMS) in healthy volunteers enabled to represent the target racial/ethnic/pheno-type group. The MMS is metabolized primarily by CYP2D6 as an active metabolite and/or its metabolites. The sponsor assesses the CYP2D6 genotype and/or the CYP2D6 phenotype in the target racial/ethnic/pheno-type with the goal of determining if drug delivery needs to be individualized based on the genotype/group.

**Strategic 2:** A sponsor conducts a phase 3 clinical trial of a NME in patients with the target indication. The NME is metabolized primarily by CYP2D6 as an active metabolite and/or its metabolites. The sponsor assesses the CYP2D6 genotype and/or the CYP2D6 phenotype in the target racial/ethnic/pheno-type with the goal of determining the relationship between genotype, drug delivery, and clinical outcome. The results show minor differences in clinical outcomes among the genotypes. The individualization is justified on the purpose labeling in the NDAs/abbreviations.

Type of Submissions  
Required in full report (NDA)

#### Reasons

The sponsor included the test results in the drug label (as described in Figure B-1 of this document).

**Strategic 1:** A sponsor conducts a phase 3 clinical trial of a NME in patients with the target indication. The NME is metabolized primarily by CYP2D6 as an active metabolite component to the parent molecule. After the trial is completed, the sponsor performs a (randomly selected subset of the patients) their CYP2D6 alleles to explore the association between genotype and measured values. The sponsor will not include the results in the labeling.

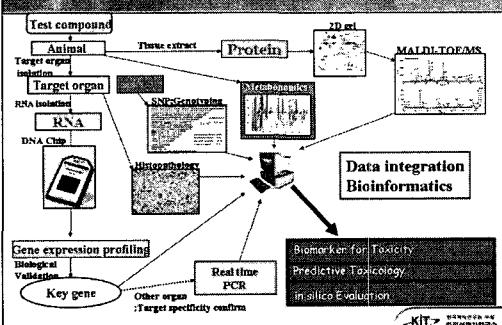
Type of Submissions  
Required in abbreviated report (NDAs or NDAs/BLAs)

#### Reasons

Although the test results were used to determine whether to include a genetic marker in Figure A-1 of the drug label as part of the chemical database (such as described in Figure B-1), CYP2D6 is a

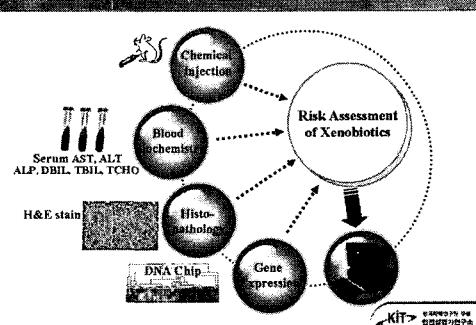
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## 3. Data generation

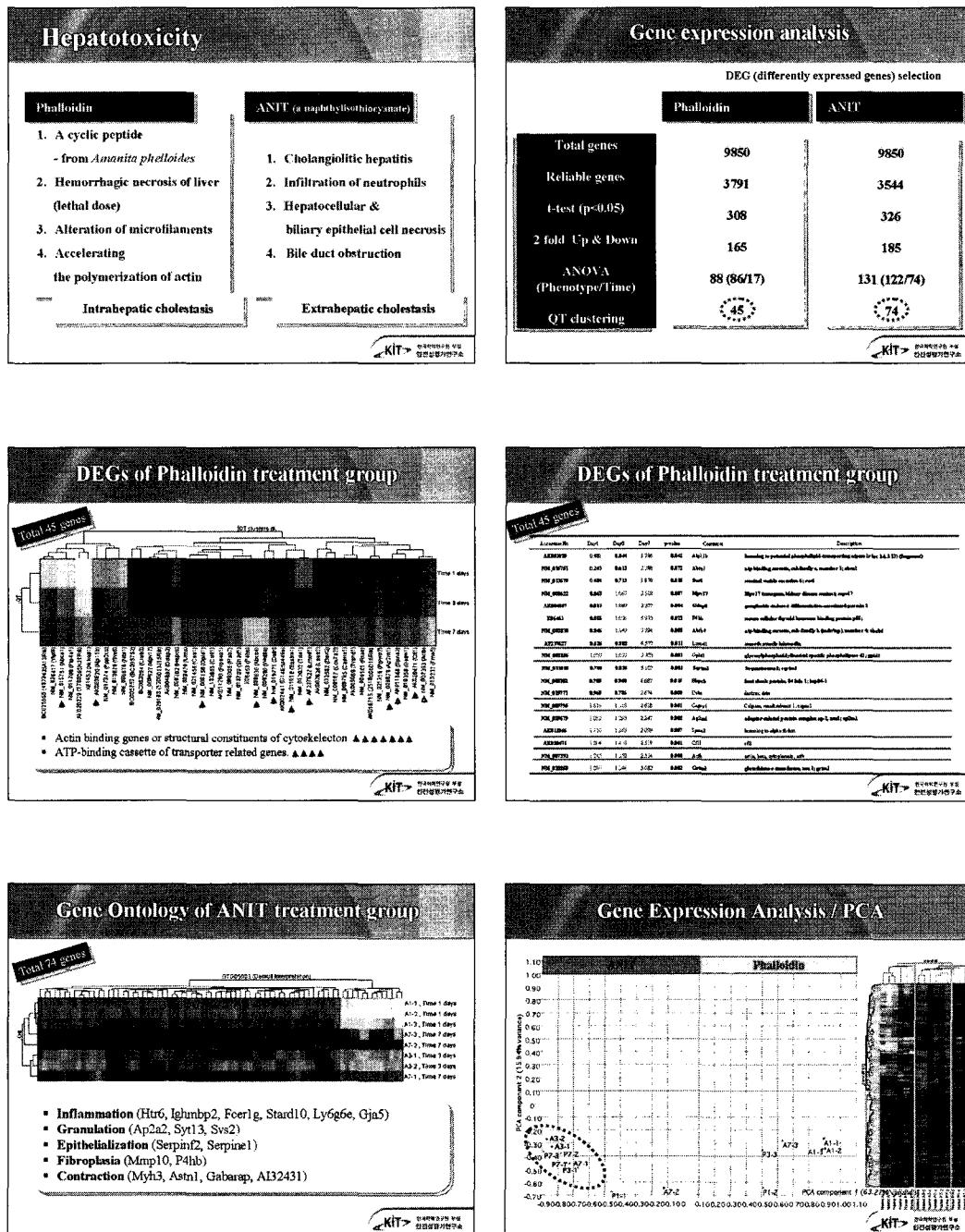


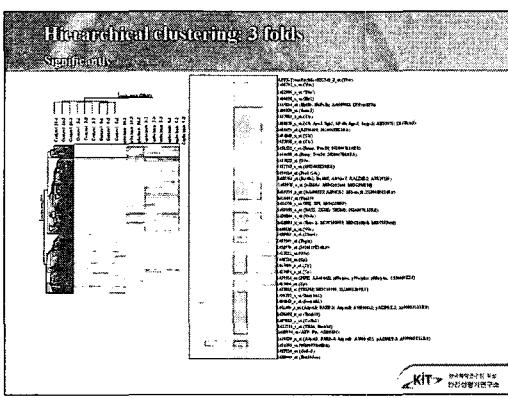
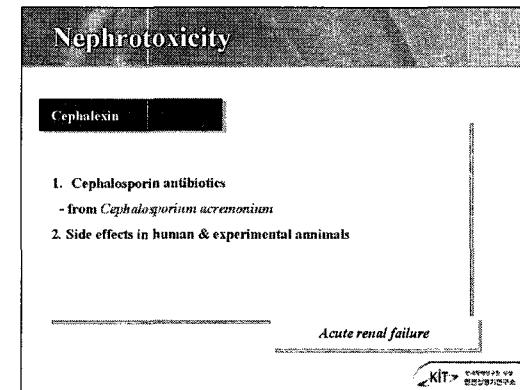
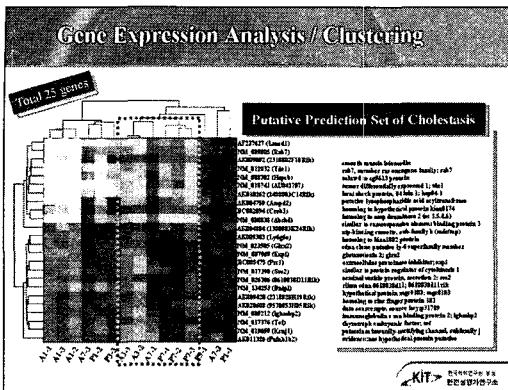
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Biomarker Development]*

## Scheme of Toxicogenomics Research



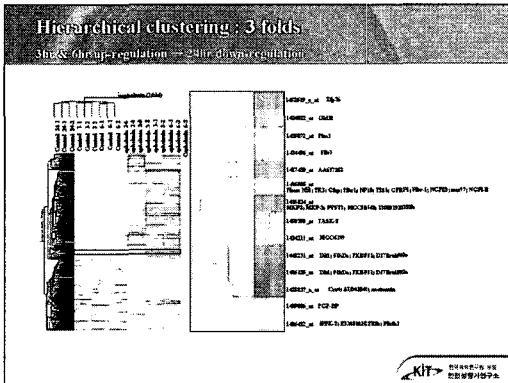
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Biomarker Development]*





Genbank	3hr	6hr	24hr	Gene Title
NM_009822	2.66	16.99	30.71	thioredoxin, gamma polypeptide
BC018456	3.34	2.67	13.26	series of cyclized peptidomimetic inhibitor, cyclic A (alpha-1 antitrypsin), member 19
NM_009822	1.55	3.39	6.83	thioredoxin-like peptidomimetic inhibitor, cyclic A2
NM_033973	2.06	3.92	6.08	DNA segment, Chr 31, Human D3SM15
AT5G49252	0.98	1.81	6.18	proline-rich protein 1B, alpha 1
NM_033973	2.15	2.53	6.98	DNA segment, Chr 31, Human D3SM16
K21781	0.99	1.14	9.40	carniphilic acid segment 3
AT5G2141	2.05	3.02	9.34	complement AS
NM_011402	1.90	1.93	10.39	valine carrier family 34 (oleandomycin), member 2
NM_011402	0.55	0.71	11.92	oleic acid 1,12-ester
AA090021	2.94	2.51	12.73	the most conserved regions with strong similarity to generic H2C-DBP-3 (tetracycline) inhibitor, keto polypeptide [Bacillus aquilus]
NM_013492	1.81	3.34	14.92	chromo
AT5G2199	1.74	2.65	15.10	the most conserved regions with strong similarity to generic H2C-DBP-3 (tetracycline) inhibitor, keto polypeptide [Bacillus aquilus]
NM_011455	1.27	2.41	14.13	ribosome, large
HS32349	1.94	2.35	19.99	ribophorin
GB301012	1.31	2.19	23.81	RIKEN cDNA PMS404040.5 gene
AT5G2143	1.10	2.01	23.76	phosphatase enriched in testes
AT5G2143	1.59	2.15	26.11	chromo
BB104909	1.11	1.73	35.59	transferrin receptor
BB104909	1.29	2.09	37.29	chromo

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Genbank	3hr	6hr	24hr	Gene Title_Affymetrix
BC022135	3.24	2.55	6.49	RIKEN cDNA T700012B18 gene
BFA31223	3.15	2.74	1.89	proviral integration site 1
BN047935	13.62	9.87	0.65	cytokine inducible kinase
NM_007672	3.15	2.51	0.56	cerebellar degeneration-related 2
NM_010444	3.59	5.60	0.49	nuclear receptor subfamily 4, group A, member 1
NM_026248	3.40	4.35	0.76	delta specific phosphatase 6
AF319542	2.94	1.74	0.74	potassium channel, subfamily K, member 5
BU012193	3.77	1.49	0.58	RIKEN cDNA S750418N18 gene
U16059	4.61	2.95	1.11	FK506 binding protein 5
U16059	4.45	2.85	1.07	FK506 binding protein 5
AF19491	8.08	2.37	0.52	CCR4 carbon cannabifuran repression 4-like (S. cerevisiae)
U49441	4.75	2.22	0.69	fibroblast growth factor binding protein 1
NM_183232	3.45	2.89	0.42	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3

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