Current Experience With Pre-Clinical Safety Biomarker Qualification

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Application and Validation of Genomic Biomarkers for use in Drug Development and Regulatory Submissions

Bethesda, October 7th, 2005

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FDA sets new standards for Development…

"Its mission is to advance and protect public health…

…by helping to <u>speed innovations</u> that make medicines more effective, safer and more affordable."

Dr. Mark McClellan, FDA Commissioner in "Improving Innovation in Medical Technology: Beyond 2002" and reported in the Pink Sheet, February 3, 2003

FDA launched the Critical Path Initiative

Novartis launched Key Strategic Initiatives

Our Goal for Regulatory Innovation:

To shape HA and industry efforts towards **the integration of new scientific technologies in drug development as the basis for regulatory decision making.**

Joerg Reinhardt

Head Development Novartis Pharma

A Genomic Safety Biomarker Validation Project

- **Goal**
  - Jointly, **FDA CDER** and **Novartis Pharma** to identify the process and analysis standards by which biomarkers for safety can be validated for use in regulatory decision-making
- **Expected deliverables**
  - Process map for validation that can be shared with academia and industry as basis for a draft guidance
  - Data regarding predictivity of specific biomarkers of renal tubular toxicity in a preclinical model
  - Proposal for applying process for validation in multiple species and man

Why Biomarkers for Nephrotoxicity?

- Current kidney functional tests are either insensitive, variable or non-specific to kidney injury.
- Approximately 2/3 of kidney function loss before BUN or creatinine increases
- BUN is highly influenced by protein metabolism
- Creatinine is influenced by muscle breakdown

Han et al., Kidney Int. 62, 2002, 237-44.

Hewitt et al, J Am Soc Nephrol. 15, 2004, 1677-89.

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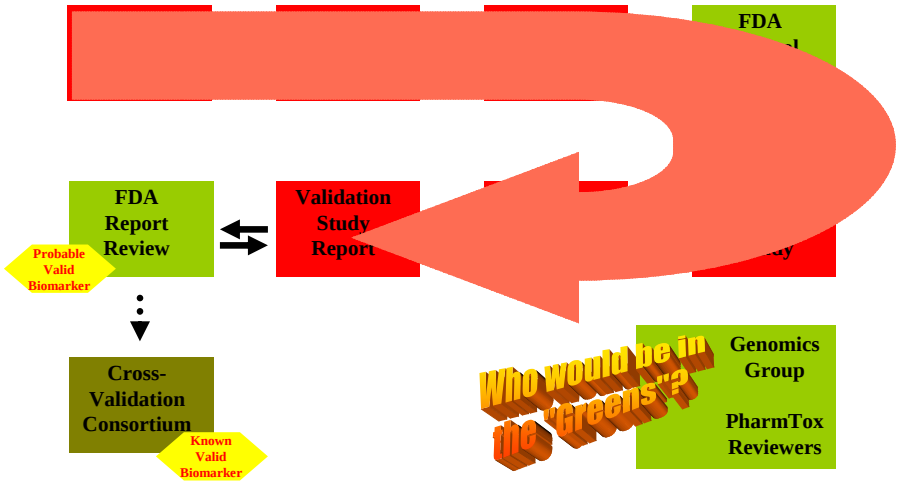
Proposed Qualification Process Map of Preclinical Safety Biomarkers



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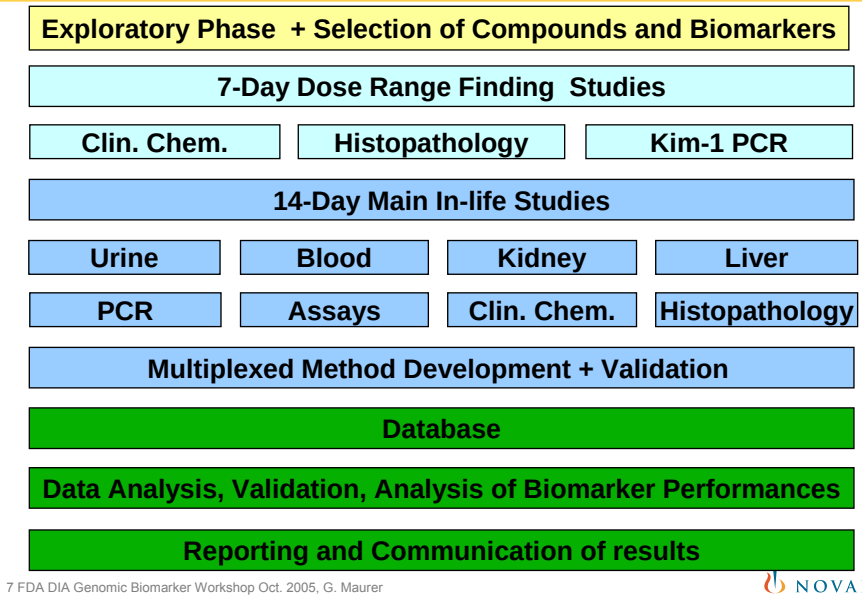
Genomic Safety Biomarker Validation Project



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Rational for Compound Selection

- Commercially available compounds
- Renal tubular toxicity achieved in the rat after treatment for up to 2 weeks
- Pathology
  - Nephrotoxicants: focus on tubular (proximal and/or distal) toxicity
  - Hepatotoxicants: different pathologies, direct toxicity
- Mixture of well and less well characterized compounds
- Different chemical classes and molecular modes of toxicity covered
- Some compounds may have additional organ toxicities beside the main target organ to evaluate the discriminative power of the approach

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8 Nephrotoxicants 2 Hepatotoxicants: Pathologies

Nephrotoxicants				
Compound	**Prox. Tub.**	**Medulla**	**Glomerul.**	**Coll. Duct**
Gentamycin	x			
Puromycin			x	
Vancomycin	x		x	
Doxorubicin	x		x	
Furosemide	x			
Litiumcarbonate	x		x	
Cisplatin	x		x	x
FK 506	x	x		
Hepatotoxicants				
ANIT (alpha-Naphtyl isothiocyanate)				
Methapyrilene				

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Rational for Biomarker Selection

- Currently well established biomarker
  - Clinical chemistry established
- New exploratory biomarker
  - Described in peer reviewed literature
  - Used in scientific community as biomarker
  - Assessment possible via protein and/or gene expression assays
  - Mechanistic relevance
  - Ideally specific for subcompartements of the nephron
- Bridge between new and currently used markers
  - Improves combination predictivity
  - Possibilities and limitations of new versus established markers

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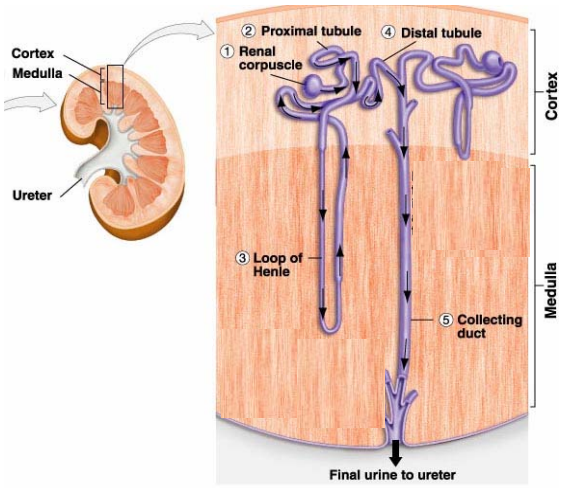
Nephron and Biomarker Localization



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Proximal Tubules
- Alpha –GST
- KIM-1
- Clusterin
- Osteopontin
- ß-2-microglobulin
- Calbindin d28
- NAG
- TIMP-1

Distal Tubules
- mu GST
- KIM-1
- Clusterin
- Osteopontin
- TIMP-1

Glomeruli
- ß-2-microglobulin
- Podocin

Vascular markers
- Cysteine-rich protein-61 (CYR61)
- VEGF

General Toxicity
- EGF
- Cystatin C
- Lipocalin

http://www.uic.edu/classes/bios/bios100/lecturesf04am/kidney01a.jpg

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Genomic Safety Biomarker Validation Project

Selection of Compounds and Biomarkers

7-Day Dose Range Finding Studies

Clin. Chem. | **Histopathology** | **Kim-1 PCR**

14-Day Main In-life Studies

Urine	Blood	Kidney	Liver
PCR	Assays	Clin. Chem.	Histopathology

Multiplexed Method Development + Validation

Database

Data Analysis, Validation, Analysis of Biomarker Performances

Reporting and Communication of results

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Study Design: In-life Phase

- Animal Model: Male Han Wistar Rats
- Dose-range finding studies
  - 3 dose groups
  - 3 animals per dose group
  - 7 days sacrifice
  - 1 control group
- Main Studies
  - 3 dose groups
  - 1 control group per study
  - 24 animals per group
  - 6 animals per group are sacrificed at
    - Day 1, Day 3, Day 7,Day 14

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Criteria for Main Study Dose Selection

Objective:

To show that the biomarkers are predictive of toxicity, it is recommended that the doses selected will result in a no effect dose, a low dose and a dose with overt toxicity

Criteria

- **Lowest dose:**
  - *no effect or bordeline dose with regard to the pathology end-points, but*
  - *expected that biomarker value slightly higher than the Ctrl.*
- **High dose:**
  - *dose with overt toxicity*
- **Mid dose :**
  - *a reasonable factor between Low and High*

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Assessments: In-life Phase

- Clinical signs:
  - Observations
  - Body weight
  - Food consumption
- Urine
  - Collected in 2 time intervals before sacrifice
  - Analysis of 14 quantitative parameters
- Blood Chemistry
  - Determination of 18 parameters
- Pathology
  - Peer reviewed pathology assessments
  - Organ weights
  - Macroscopic
  - Microscopic

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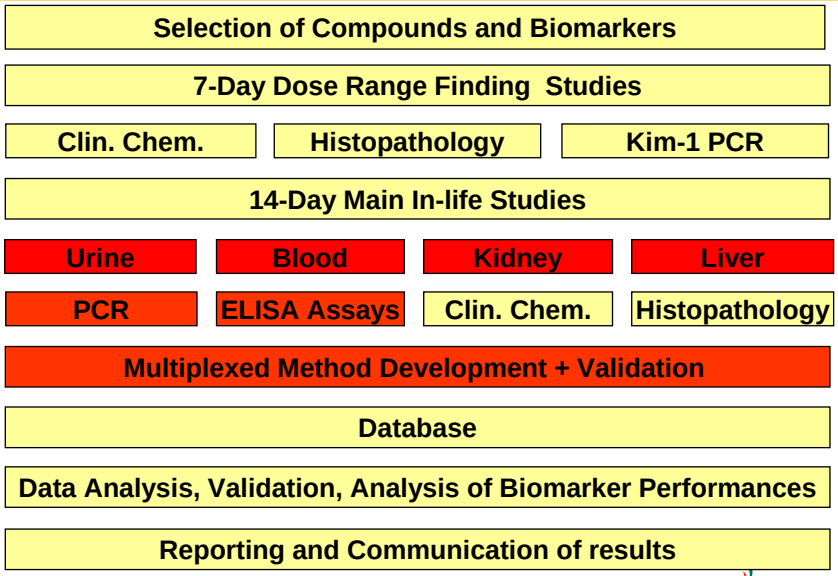

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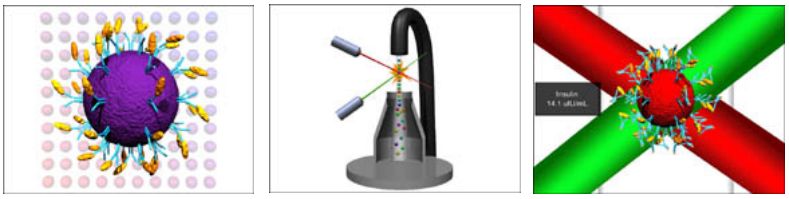
Methods: Analytical Methods

- Analytical methods are validated according to regulatory guidelines
- mRNA
  - Extracted from Blood, Kidney and Liver
  - Measured by PCR (PCR card)
  - Stable housekeeping genes selected with dose range finding studies
- Proteins
  - Measured in urine, plasma, tissue extracts
  - Multiplexed assays based on microspheres (sample volume issue)



http://www.rulesbasedmedicine.com

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Status of ELISA Multiplexed Assays

- 4 assays available
  - Alpha glutathione-S-transferase (α-GST), Tissue inhibitor of metalloproteinases-1 (TIMP-1), Epidermal growth factor (EGF), Vascular endothelial growth factor (VEGF)
- 3 assays in late stage of development
  - Cystatin C, Osteopontin, Clusterin
- Antibodies and antigens for 7 assays in development
  - ß-2-microglobulin, N-acetyl-b-D-glucosaminidase (NAG), Calbindin d28, Cysteine-rich protein-61 (CYR61), Glutathione-S-transferase Yb1 (GST Yb1, mu GST), Kidney injury molecule-1 (Kim-1), Neutrophil lipocalin (HNL, Lipocalin 2) and Podocin

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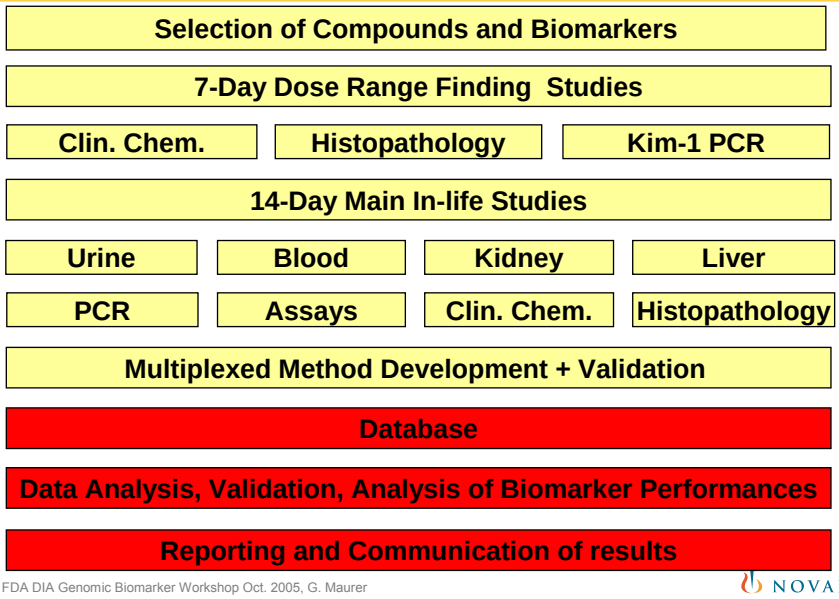

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Mining of Data and Interpretations 1/2

- Reference data: Histopathology and clinical chemistry parameters
- Models are built and optimized with calibration data, performance is tested by validation data set
- Assesment of predictive value of individual biomarkers and biomarker patterns
  - Find an optimal model for the prediction of toxicity classes
  - Anticipated sensitivity / specificity > 95% compared to histopathology
- Assessment of predictive value for chronic toxicity
  - Find optimal model, which predicts toxicity class for later time points

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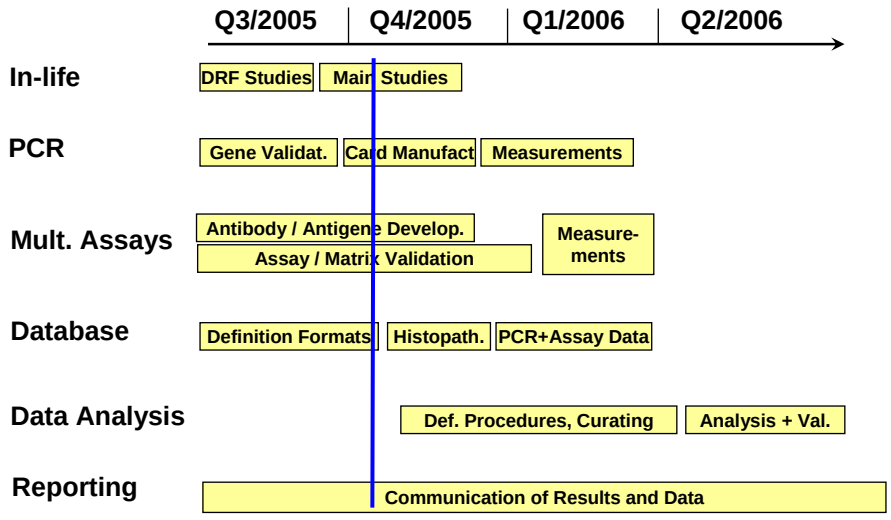
Mining of Data and Interpretations 2/2

- Parameters accessed for models:
  - Sensitivity, also compared to histopathology and clinical chemistry
  - Selectivity
    - Versus hepatotoxicity and other organ toxicities
    - Subtypes of nephrotoxicity
  - Reproducibility and Robustness
    - Between animals
    - Between compounds: analysis of differences in time dependence
    - Reproducibility between time points
- Analysis of matrices
  - Comparison of proteins in urine and plasma
  - Comparison of gene expressions in liver, kidney and blood (possibly also urine)
  - Correlation of gene expressions and protein concentrations

Methods: Database and Data Analysis

- **Database**
  - Stores all data generated by the project.
- **Data analysis**
  - Univariate (wide-spread methods, interpretable by non-experts)
  - Multivariate (combination of biomarkers, identification of patterns and similarities...)
  - Model-based (robust)
  - Non-model-based methods (e.g. do not rely on linearity)
  - Non-supervised (allow unbiased analysis)
  - Supervised (often more powerful)
  - Feature selection (identification of significant biomarkers)

Genomic Safety Biomarkers Validation Project: Timelines



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Outlook

- **Limitations of the current concept**
  - Treatment duration: Up to 2 weeks versus chronic treatment
  - One species: can we extrapolate our results to other animal models?
  - Biomarkers in animal models and their predictivity for humans.
- **Inter-species validation**
  - Samples from clinical trials
  - Mining of existing data sets
- **Inter-laboratory validation**
  - Cross-validation with other partners i.e academia, pharmaceuticals , ...
  - Cross-validation with other analytical methods
- **Sharing this experience with others**
  - Consortium
  - Publications

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What do we get out .... Conclusions

1. **A qualification process**
   - structures the flow of interaction, clarifies roles and expectations
2. **RNA, Protein Assays**
   - to quantify BM in either rat kidney tissue, urine or plasma
3. **Sensitive, predictive BMs relevant for the rat kidney function**
   - One BM, a profile of BM;
4. **Pharmacogenomic test**
   - a description for applicability of the assays
   - know probable BM
5. **Safer Drugs: only achieved if BM has been qualified in man**
   - preclinically: extend the use to more animal species
   - inter-laboratory, cross validation, role of a consortium
   - achieve status of Known valid Biomarker

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15 Biomarkers

- ß-2-microglobulin*
- N-acetyl-b-D-glucosaminidase (NAG)
- Glutathione-S-transferase - Alpha (alpha-GST)
- Glutathione-S-transferase Yb1 (GST Yb1, mu GST)
- Calbindin d28
- Clusterin
- Cystatin C
- Cysteine-rich protein-61 (CYR61)
- Epidermal growth factor (EGF)
- Kidney injury molecule-1 (Kim-1)
- Neutrophil lipocalin (HNL, Lipocalin 2)
- Osteopontin
- Podocin
- Tissue inhibitor of metalloproteinases-1 (TIMP-1)
- Vascular endothelial growth factor (VEGF)

* **only protein**

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