Based on these recommendations, we refined our initial case-study approach to produce a generalizable approach that can be used to evaluate genomic data in new chemical assessments.

1.4. RESEARCH NEEDS

The following research needs could potentially improve the utility of genomic data in risk assessment:

- Perform parallel toxicity and toxicogenomic studies with similar design characteristics (i.e., dose, timing of exposure, organ/tissue evaluated) in order to obtain comparable results which would aid our understanding of the relationship between gene expression changes and phenotypic outcomes.

- Test multiple doses, with increased numbers of animals, in microarray and toxicity studies (see bullet above) in order to relate the dose to the gene expression and pathway response, and to the *in vivo* response.

- Perform a time-course global gene expression study over a relevant exposure interval (e.g., critical window of development) in order to identify the earlier and possibly, initiating gene expression events.

- Generate TK data in an appropriate study (e.g., time, dose, tissue), and obtain a relevant internal dose measure to derive the best internal dose metric.

- Further develop bioinformatic methods for analyzing genomic data for the purpose of use in risk assessment.

As a result of considering how to best use genomic data in risk assessment, we identified a number of issues for future consideration. As more and various types of genomic studies are performed, genomic data will likely inform multiple steps of the risk assessment process beyond MOA. To facilitate the advancement of the use of genomics in risk assessment, first, we need approaches to utilize genomic data quantitatively, specifically, the application of genomic data to dose-response, intraspecies variability, and TK. Second, analytical methods tailored to use in risk assessment are needed. Bioinformatics methods development work, some initiated in this project, continues to evolve. The goal is to develop and/or adapt existing bioinformatic tools currently used for hypothesis generation to the express purpose of utilizing genomic data for risk assessment. The pathway activity level method presented in this report is one promising approach for application to risk assessment. However, continued efforts, with input from both statistical modeling and biology experts, is required to validate, test, and refine these methods.

Third, training risk assessors in genomic data analysis methods would assist EPA in the evaluation and interpretion of complex, high-density data sets and in performing new analyses when necessary.

Finally, some of the issues in utilizing genomic data in health and risk assessment are not unique to genomic data but apply to precursor event information in general. Two of these issues are (1) defining adversity and (2) establishing biological significance of gene expression changes or pattern. The design and performance of appropriate studies, with both genomic and toxicity components, may help to address the scientific aspects of these two important issues (see research needs above).

To the best of our knowledge, this is the first systematic approach for using genomic data in health assessment at EPA. We believe that this report can be used by risk assessors when considering a large range of potential applications, issues, and methods to analyze genomic data for future assessments. This approach advances efforts in the regulatory and scientific communities to devise strategies for using genomic data in risk assessment, and it is consistent with the pathway-based risk assessment vision outlined in the National Research Council's (NRC's) report, *Toxicity Testing in the 21st Century*. We also anticipate that the research needs and future considerations described herein will advance the design of future toxicogenomic studies for application to risk assessment, and as a result, benefit the bioinformatic, toxicogenomic, and risk assessment communities.

Prediction of ***liver toxicity*** in the animal study using the mechanistically relevant *in vitro* screening assay data

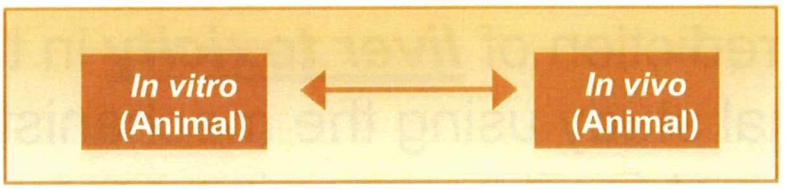
Weida Tong, Ph.D
Director of Center for Toxicoinformatics
Division of Systems Toxicology, NCTR/FDA

Drug Induced Liver Injury (DILI)

- Only 20% of patients with acute liver failure because of DILI survive with supportive care
- 1% patients develops DILI during the course of hospitalization
- DILI is the single most frequent reason for drug withdraw and "black-box" warning
  - DILI has been linked to nearly 1000 drugs
- Acetaminophen accounts for 46% incidences of acute liver failure
  - Trend: 28% in 1998 to 51% in 2003
- DILI is more common in females for reason unclear
  - Women accounts for 79% of APAP-related DILI and 73% of idiosyncratic drug reaction

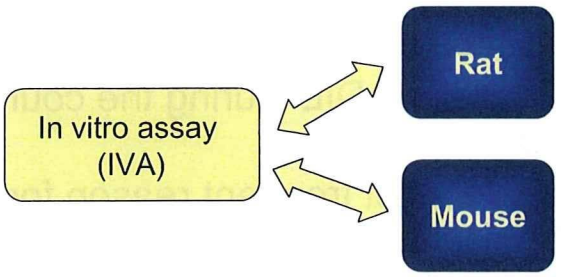
Larson et al. Hepatology 2005, 42:1364-1372
Ostapowicz and Lee, J. Gast – Roenterol Hepatol 2000, 15: 480-488
Ostapowicz et al. JMAM 1998: 279:1200-1205

ToxCast Data



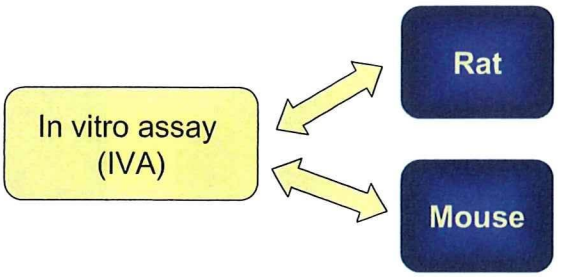
- Two types of data
  - *In vitro* assay (IVA) data
  - Calculated descriptors
- Liver tox endpoints for both *rat* and *mouse*
  - Hypertrophy
  - Necrosis
  - Proliferation Lesions
  - Tumors

Study Design and Objectives



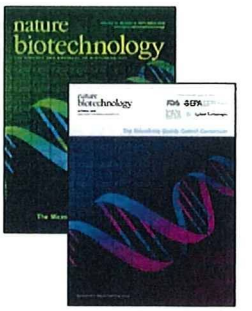
- Comparative analysis between the rat and mouse models
  - The performance of the rat models vs mouse models for each endpoints
  - Which IVAs contribute most to these models
  - Are the same features used in both species

The Choice of Machine Learning



- Soft Independent Modeling of Chemical Analogy (SIMCA)
- Artificial Neural Network (ANN)
- K-Nearest Neighbor (KNN)
- Decision Tree
- Support Vector Machines (SVMs)
- Fisher's Linear Discriminant (FLD)

The FDA MicroArray Quality Control (MAQC) Project



Feb 2005

MAQC-I — **17 months**

- MAQC-I: Technical Performance
  - Reliability of microarray technology
  - Cross-platform consistency
  - Reproducibility of microarray results
  - 137 participants from 51 organizations

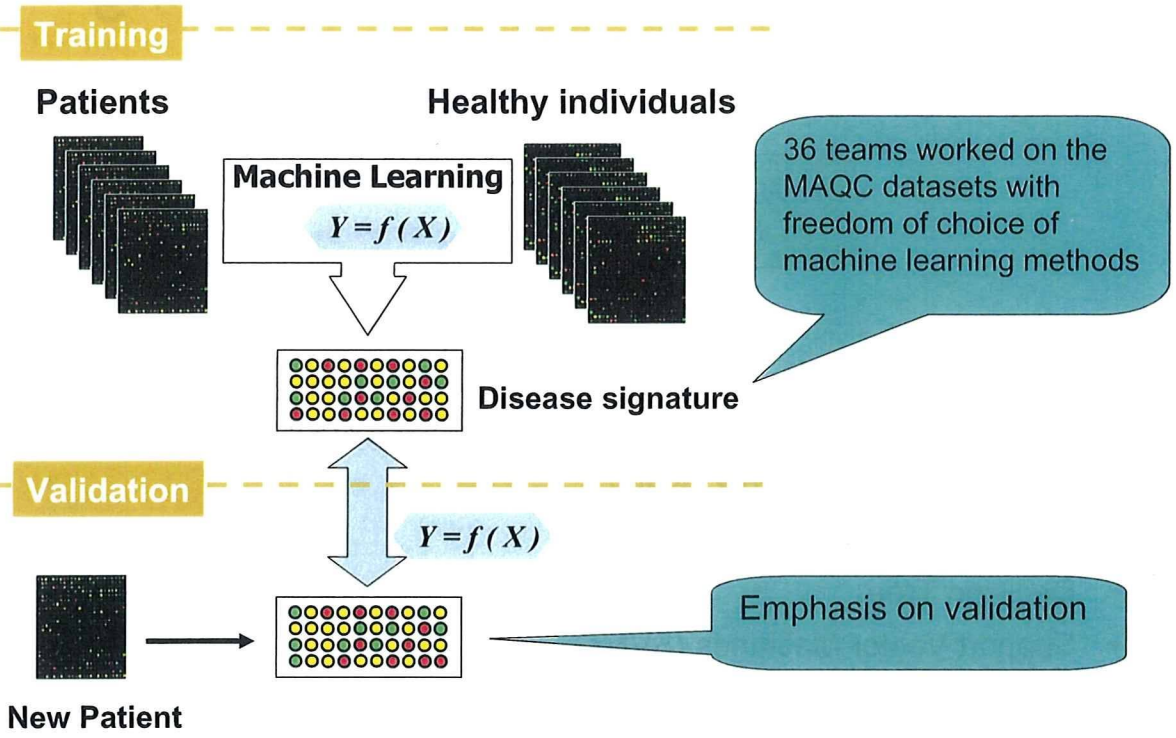
Sept 2006

MAQC-II — **30 months**

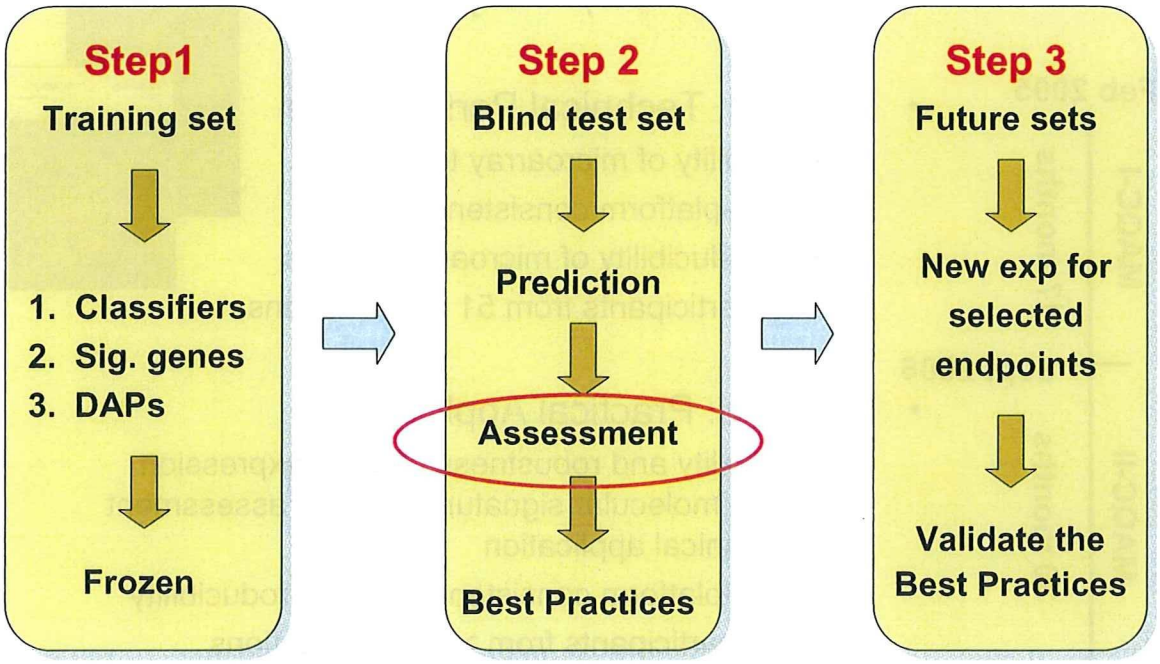
- MAQC-II: Practical Application
  - Reliability and robustness of gene expression based molecular signatures for risk assessment and clinical application
  - Cross-platform consistency and reproducibility
  - >200 participants from >50 organizations
  - 21 manuscripts were submitted to Nat Biotech

Mar 2009

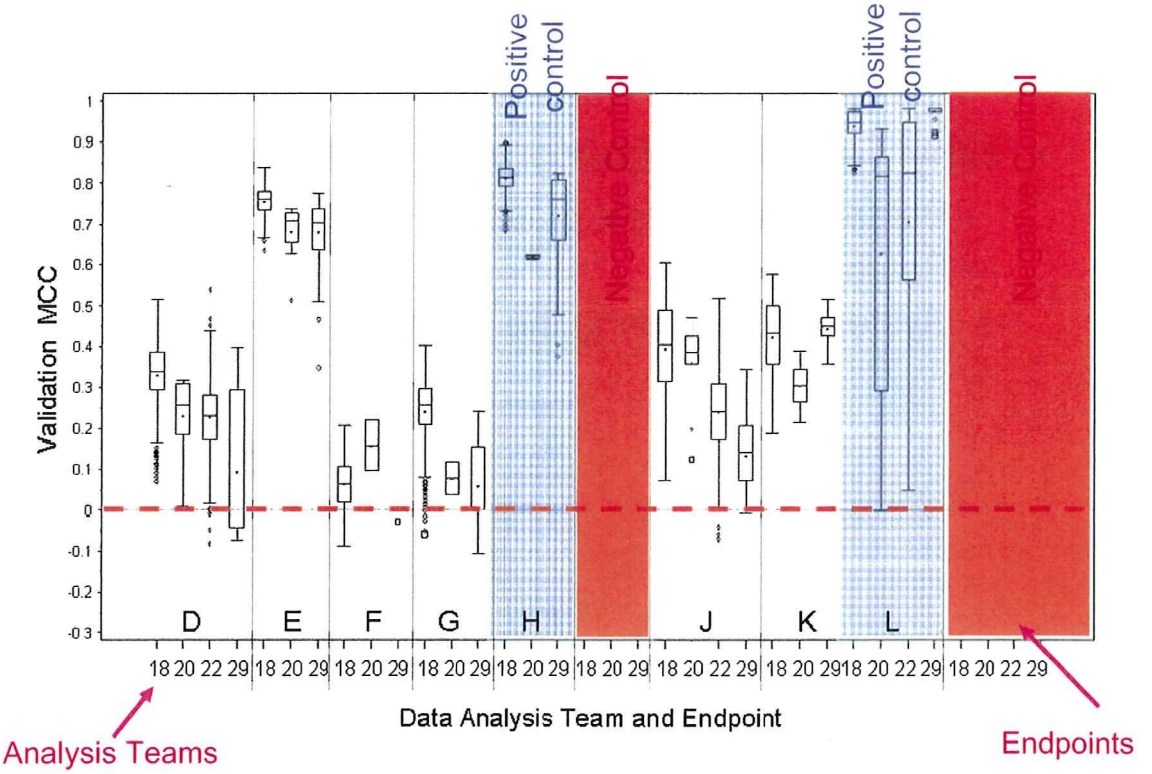
MAQC Strategy

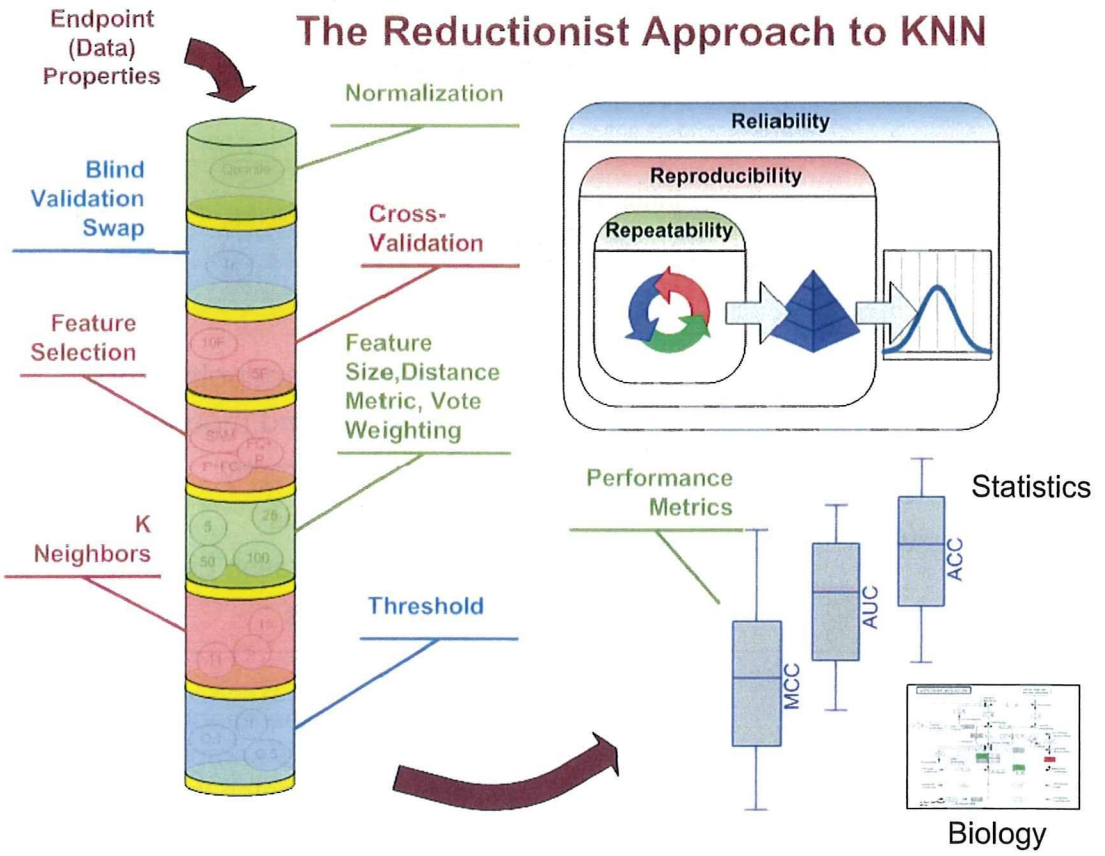


Three-Step Approach



MAQC-II KNN Results



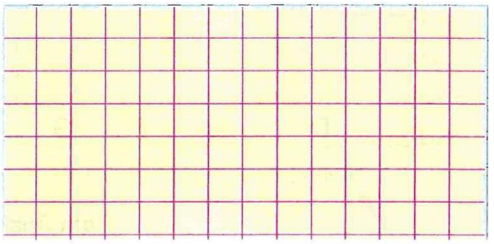


Lessons Learned From MAQC-II for KNN Through Systematic Evaluation

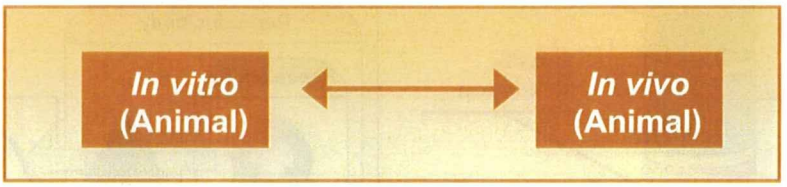
- Why Team18 outperformed other teams:
  - Balance the sample classes
  - Batch correction
  - Systematic exploring the *k*-feature space

# of features

k



Two Key Data Preprocessing



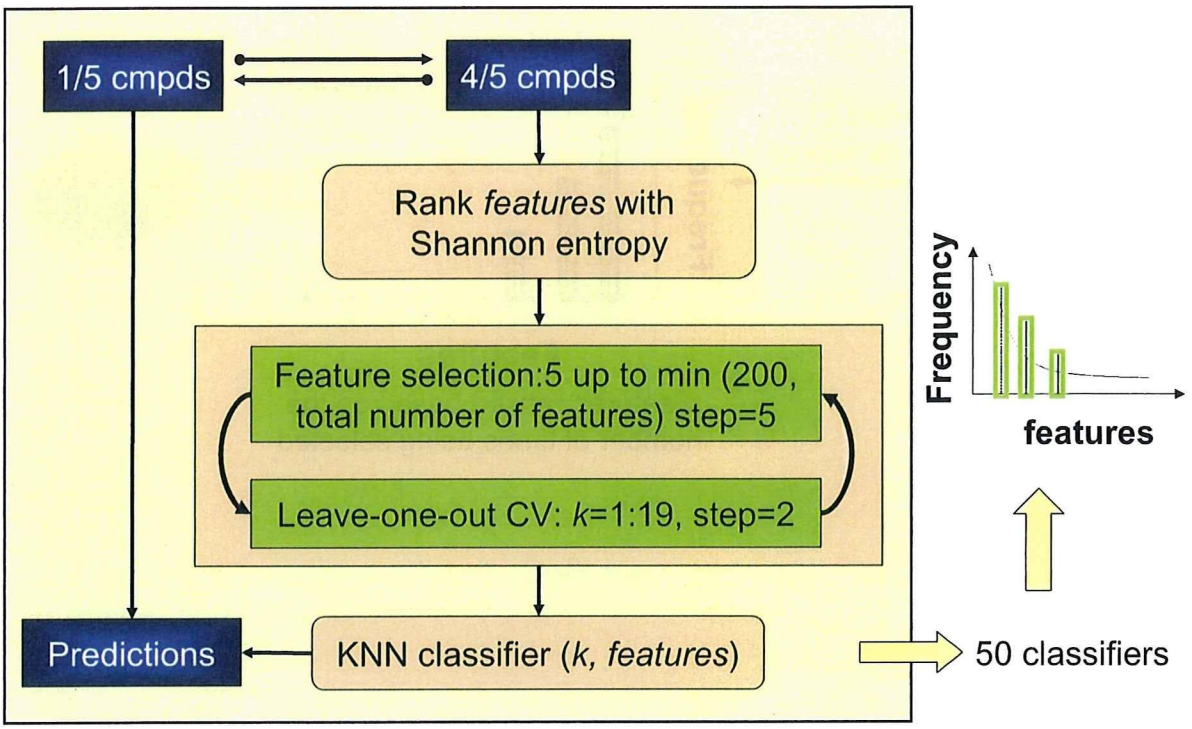
- Two types of data
  - *In vitro* assay data (binary)
  - Calculated descriptors (continuous)

Remove the "batch effect" by only including IVA

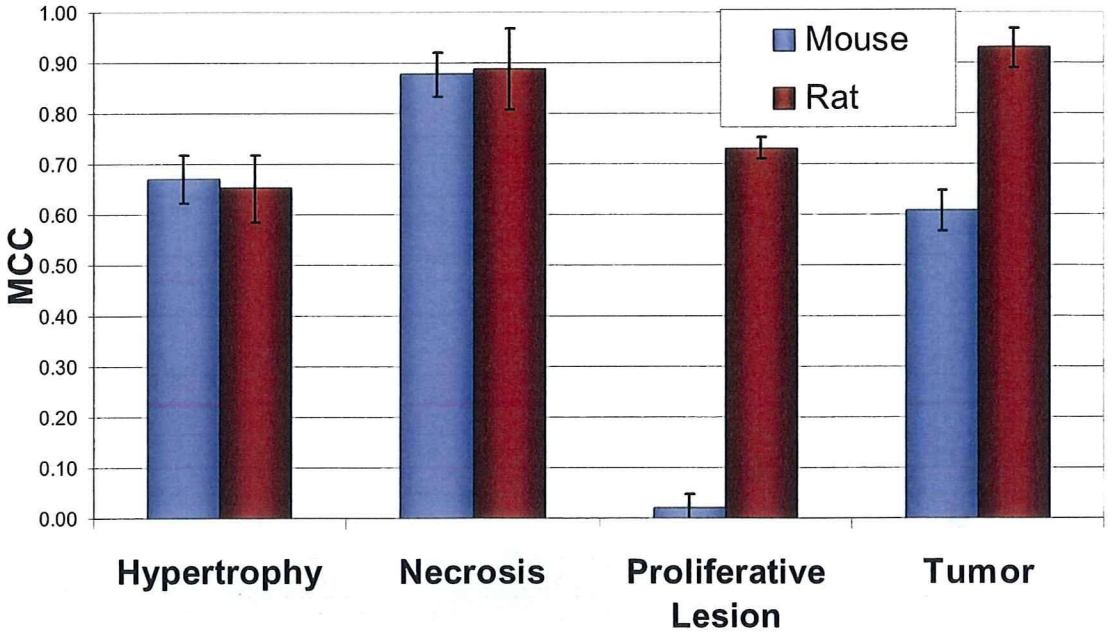
- Liver tox endpoints for both rat and mouse
  - Hypertrophy
  - Necrosis
  - Proliferation Lesions
  - Tumors

Over-sample the minority class by multiplying its sample size to approximately match the number of samples of the majority class

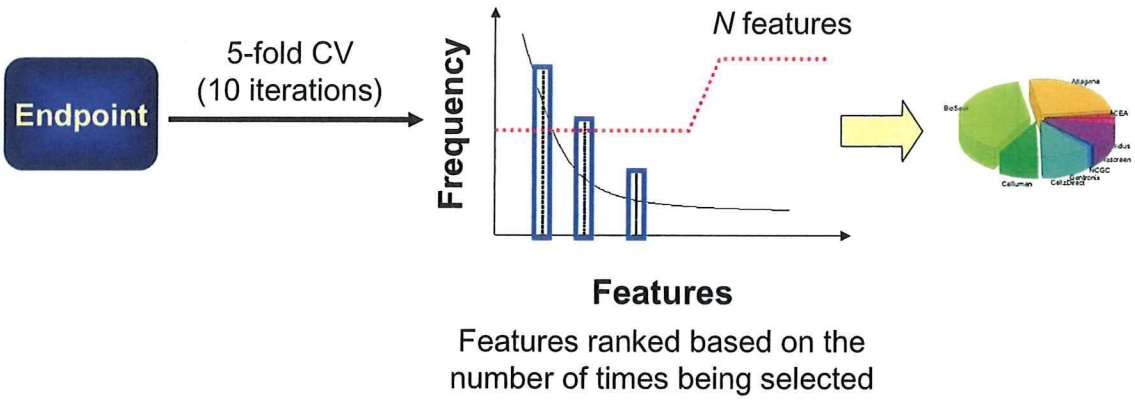
5-Fold Cross-Validation (10 iterations)



5-Fold Cross-Validation Results (MCC)



Which IVAs Contribute Most to The Predictive Models?

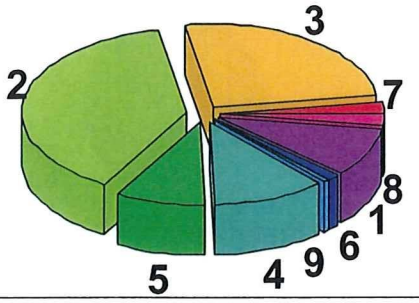
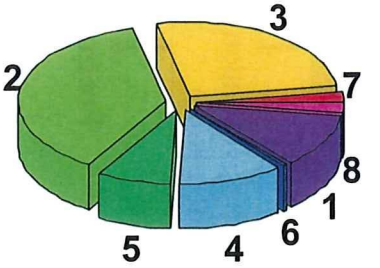
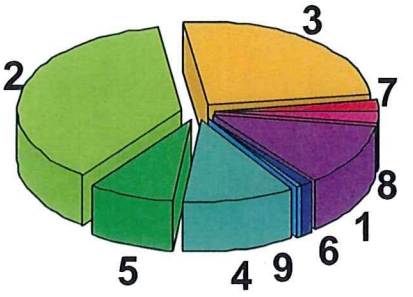
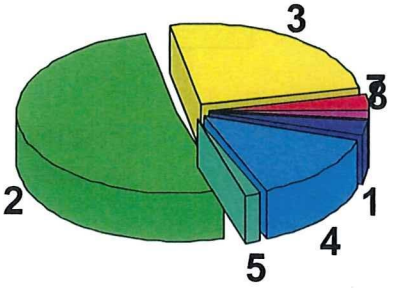


Features ranked based on the number of times being selected

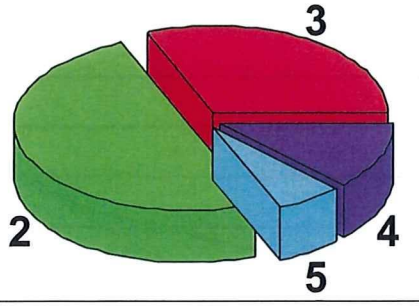
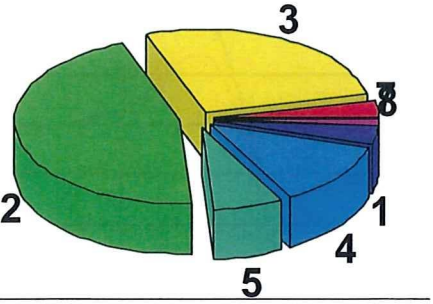
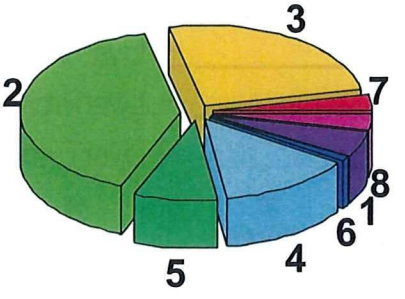
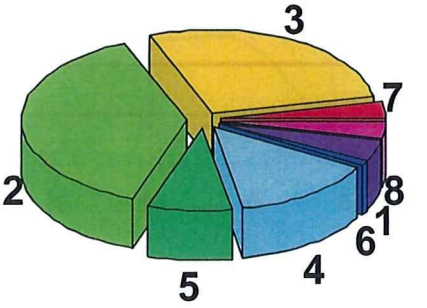
In Vitro Assays (IVAs)

	Assays	# of features
1	Novascreen	239
2	BioSeek	87
3	Attagene	81
4	CellzDirect	48
5	Cellumen	33
6	NCGC	24
7	ACEA	7
8	Solidus	4
9	Gentronix	1
	sum	**524**

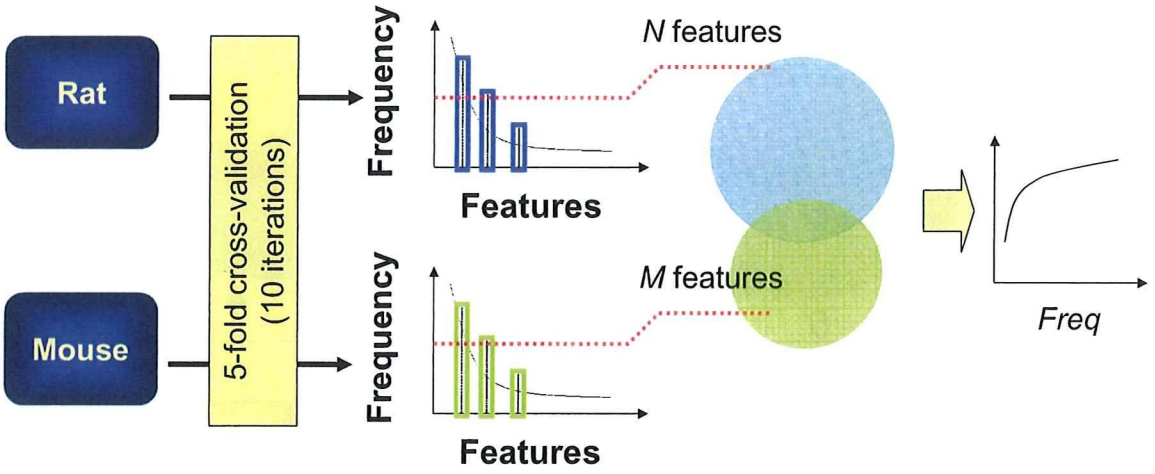
Distribution of IVA Features (Freq >= 40) - Rat vs Mouse

	Hypertrophy	Necrosis
Mouse		
Rat		

Distribution of IVA Features (Freq >= 40) - Rat vs Mouse

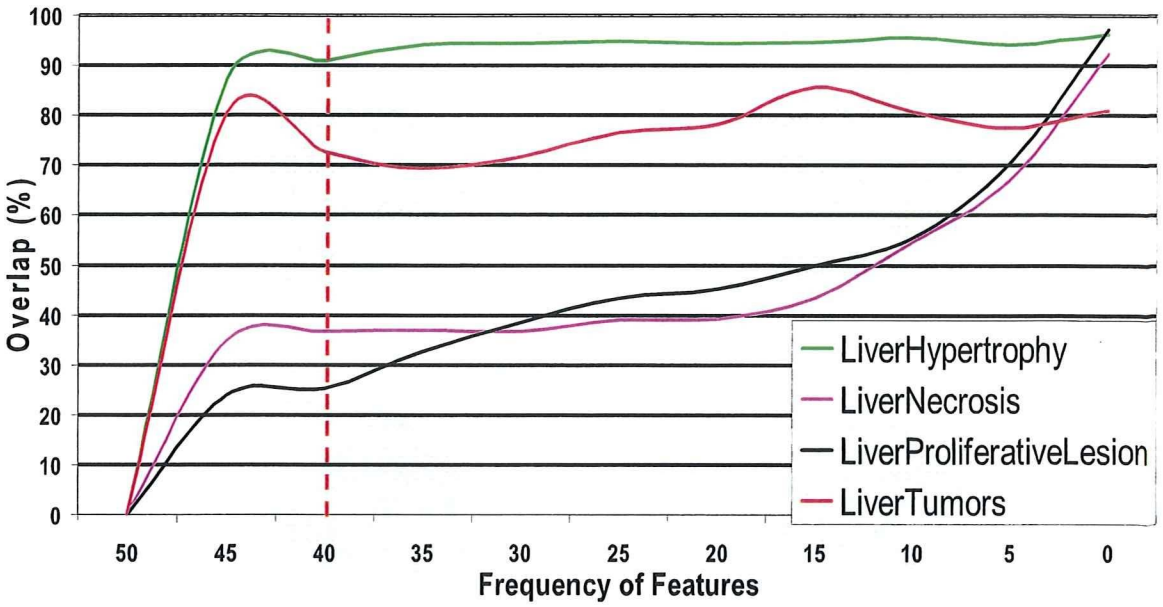
	Proliferative Lesions	Tumors
Mouse		
Rat		

How much overlap in feature between the rat and mouse models

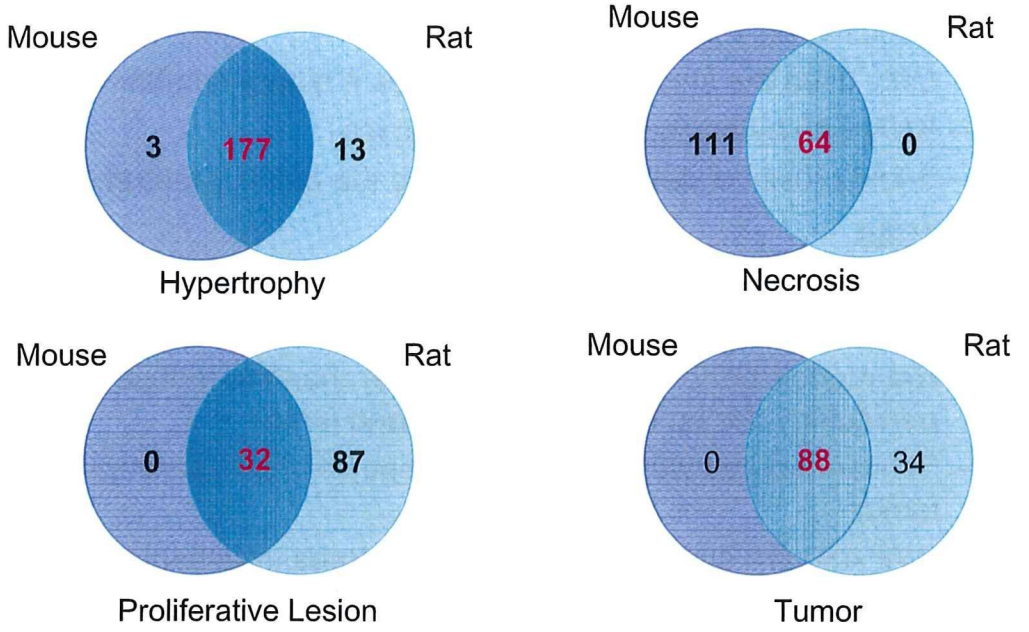


Features ranked based on the number of times being selected

Overlap of Features



Overlap of IVA Features



Common *IVA Features* (Freq>40)

Hypertrophy	Necrosis	ProliferativeLesion	Tumor
Solidus_AllEnzyme	Solidus_P450	CLZD_CYP2B6_24	Solidus_P450
Solidus_NoEnzyme	NVS_ADME_hCYP2C19	CLZD_CYP2B6_48	NVS_ADME_hCYP2C19
Solidus_P450	NVS_NR_hPXR	CLZD_CYP2B6_6	NVS_MP_rPBR
Solidus_PhaseII	CLZD_CYP1A1_24	CLM_CellLoss_72hr	NVS_NR_hPXR
NVS_ADME_hCYP1A2	CLZD_CYP1A1_6	CLM_MitoticArrest_72hr	CLZD_CYP1A1_24
NVS_ADME_hCYP2B6	CLZD_CYP1A2_24	BSK_3C_Proliferation	CLZD_CYP1A1_48
NVS_ADME_hCYP2C18	CLZD_CYP2B6_24	BSK_BE3C_uPAR	CLZD_CYP1A1_6
NVS_ADME_hCYP2C19	CLZD_CYP2B6_48	BSK_hDFCGF_CollagenIII	CLZD_CYP1A2_24
NVS_ADME_hCYP2C9	CLZD_CYP2B6_6	BSK_hDFCGF_MMP1	CLZD_CYP1A2_48
NVS_ADME_hCYP3A5	CLZD_CYP3A4_24	BSK_hDFCGF_Proliferation	CLZD_CYP2B6_24
NVS_ADME_rCYP2A1	CLZD_CYP3A4_48	BSK_hDFCGF_VCAM1	CLZD_CYP2B6_48
NVS_ADME_rCYP2B1	CLM_MitoticArrest_72hr	BSK_LPS_PGE2	CLZD_CYP2B6_6
NVS_ADME_rCYP2C11	BSK_3C_hLADR	BSK_SAg_CD38	CLZD_CYP3A4_24
NVS_ADME_rCYP2C6	BSK_3C_MCP1	BSK_SAg_CD40	CLZD_CYP3A4_48
NVS_ADME_rCYP2D2	BSK_3C_Proliferation	BSK_SAg_CD69	CLZD_HMGCS2_48
NVS_ADME_rCYP3A1	BSK_3C_Vis	BSK_SAg_Eselectin	CLM_CellLoss_72hr
NVS_ADME_rCYP3A2	BSK_4H_MCP1	BSK_SAg_PBMCCytotoxicity	CLM_MicrotubuleCSK_72hr
NVS_MP_hPBR	BSK_4H_VCAM1	BSK_SAg_Proliferation	CLM_MitoMass_24hr
NVS_MP_rPBR	BSK_BE3C_hLADR	BSK_SM3C_Proliferation	CLM_MitoMembPot_1hr
NVS_NR_hPXR	BSK_BE3C_IP10	ATG_Ahr_CIS	CLM_MitoticArrest_72hr
NCGC_PXR_Agonist_human	BSK_BE3C_uPA	ATG_AP_1_CIS	CLM_OxidativeStress_72hr
NCGC_PXR_Agonist_rat	BSK_BE3C_uPAR	ATG_BRE_CIS	BSK_3C_hLADR
xT.xTID..4.dim.xT..2..	BSK_hDFCGF_CollagenII	ATG_CMV_CIS	BSK_3C_MCP1
CLZD_ABCB11_48	BSK_hDFCGF_IP10	ATG_ERa_TRANS	BSK_3C_Proliferation

Summary

- Approaches
  - Focused on 4 liver tox endpoints
  - Only used the *in vitro* data (treated as a binary number 0/1)
  - KNN with balancing the sample classes
  - 5-fold cross-validation with exploring the entire k-feature space
- Results
  - Good models were obtained for all the endpoints for both species except for the mouse proliferative lesions
  - Rat models generally performed better than the mouse models, particularly, for proliferative lesions and tumor
  - Similar features were used in both species for hypertrophy and tumor
  - CYP 450 are among the important features (mechanistically informative IVA endpoints)

Acknowledgement

- Zhenquiang Su
- Huixiao Hong

Biomarker Qualification Process

DRUG INFORMATION ASSOCIATION

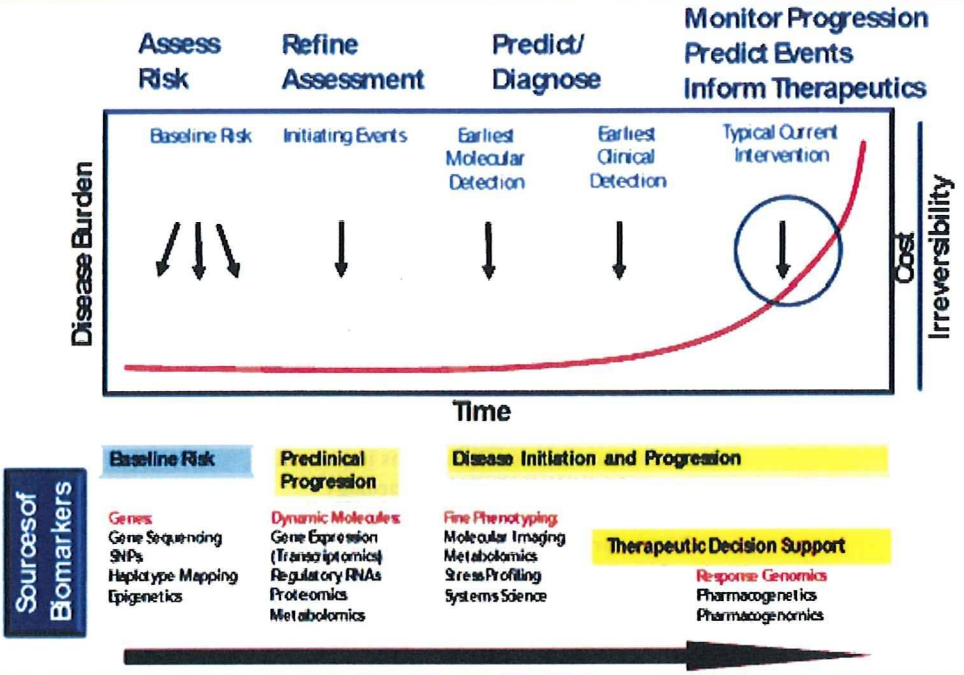
Dedicated to your information and advancement.

Federico Goodsaid, Ph.D.
Associate Director for Operations in Genomics
Office of Clinical Pharmacology
Office of Translational Science
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
10903 New Hampshire Avenue, Building 51, Room 2148
Silver Spring, MD 20903-0002
phone: 301-796-1535
email: Federico.Goodsaid@fda.hhs.gov

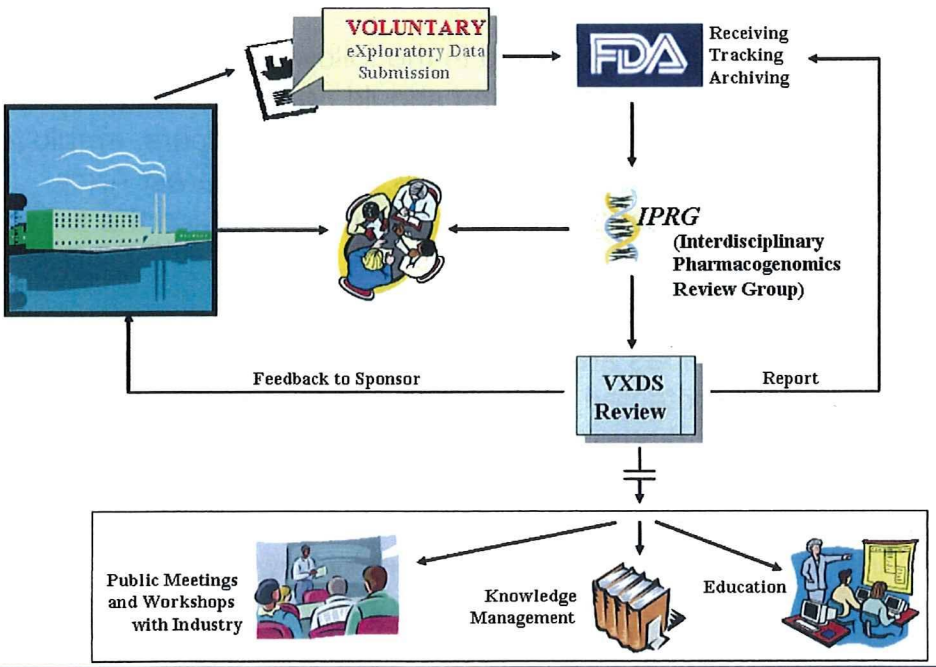
Disclaimer

The views and opinions expressed in the following PowerPoint slides are those of the individual presenter and should not be attributed to Drug Information Association, Inc. ("DIA"), its directors, officers, employees, volunteers, members, chapters, councils, Special Interest Area Communities or affiliates, or any organization with which the presenter is employed or affiliated.

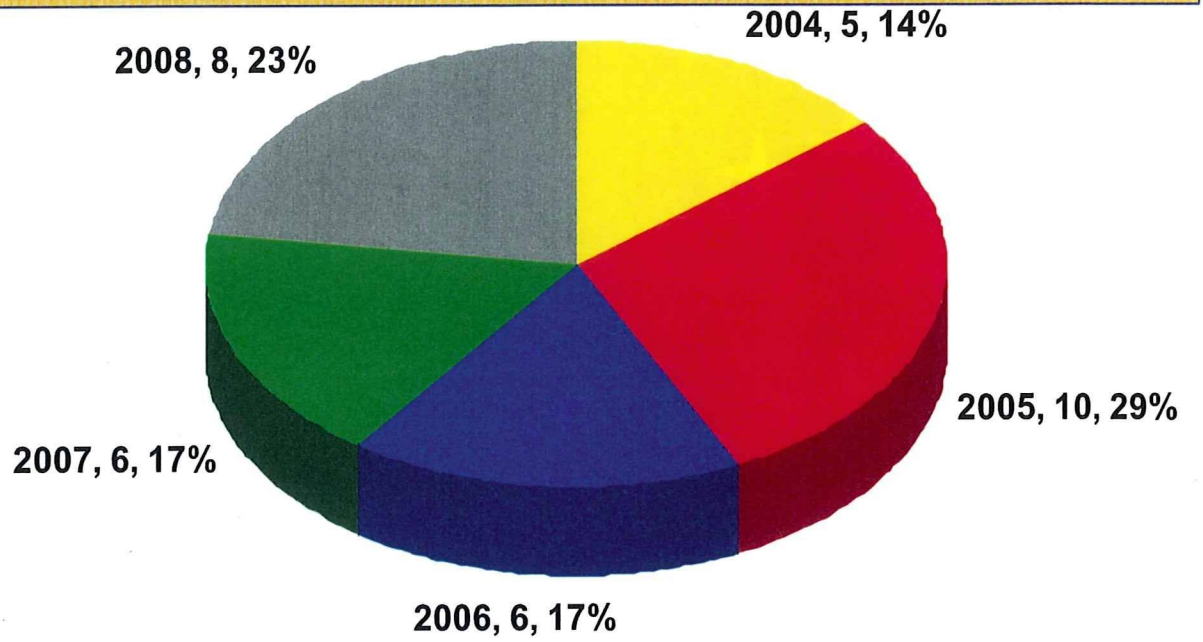
Biomarker Entry Points in Drug Development



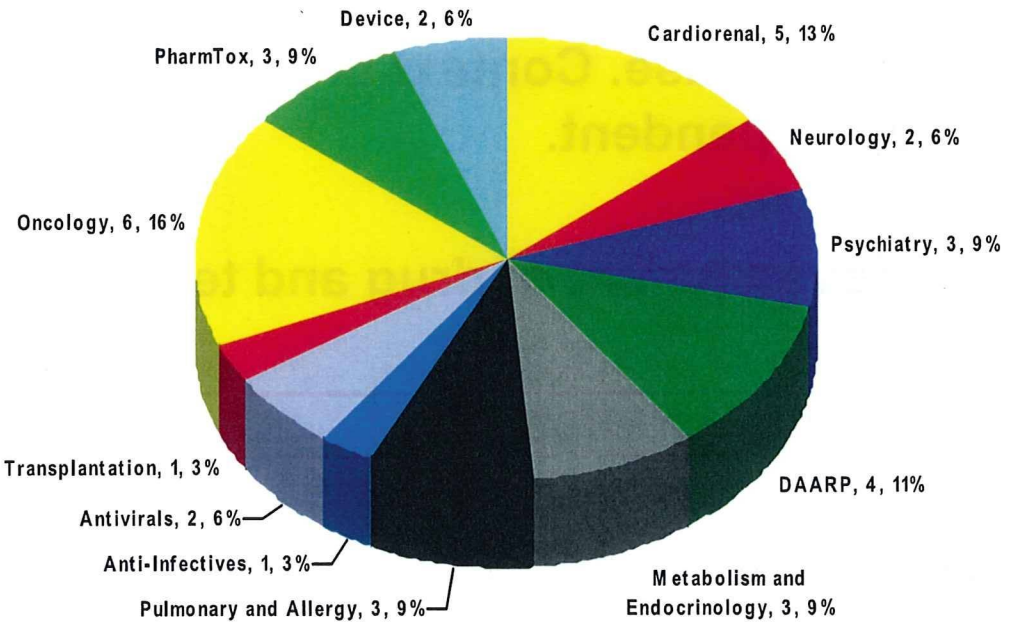
Introduction of Exploratory Biomarkers: *Voluntary eXploratory Data Submissions*

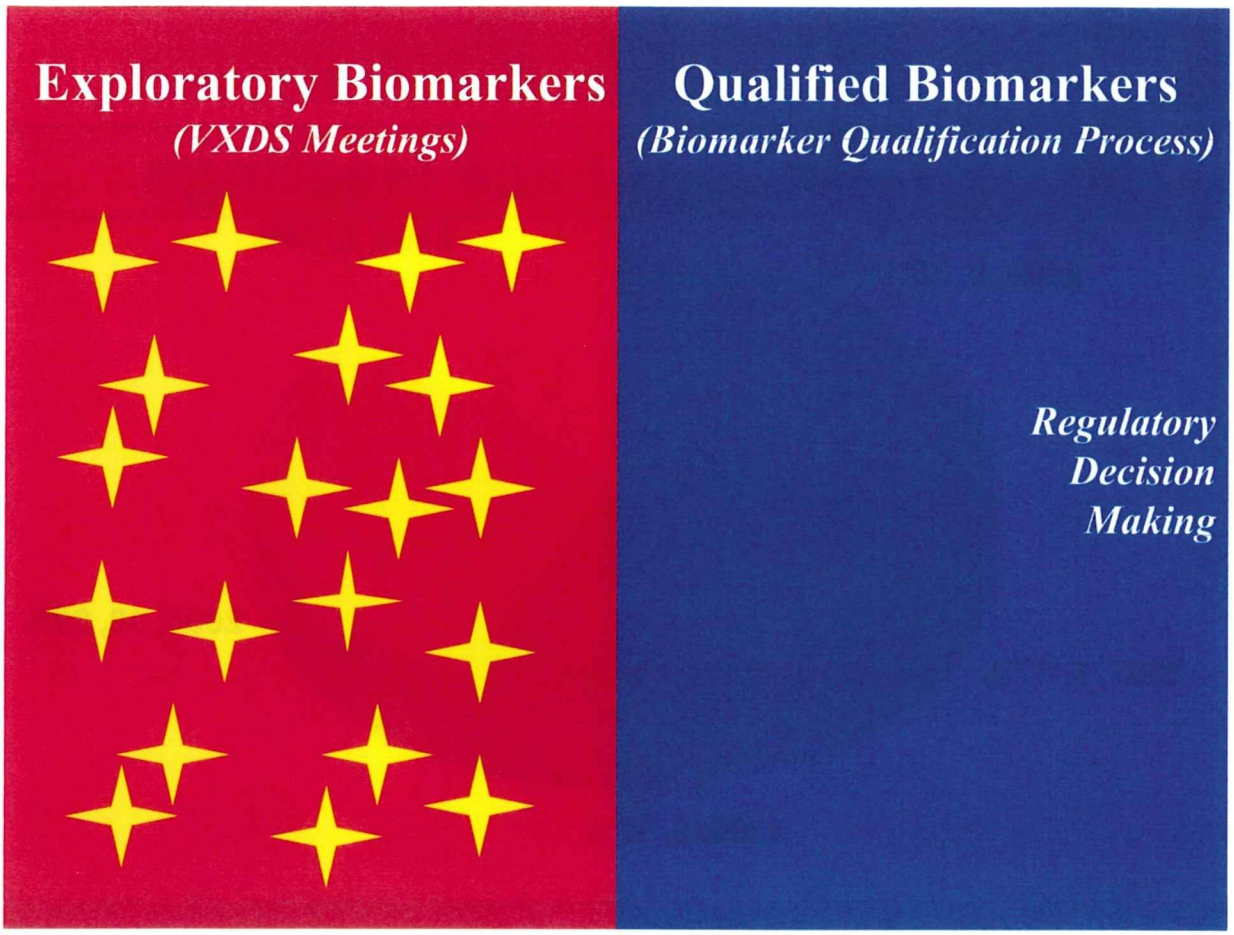


VXDS Face-to-Face Meetings



VXDS Clinical Divisions





DIA
Drug Information Association

How do we qualify biomarkers today at the FDA?

- Case-by-case. Context of use *always* drug-dependent.
- Codevelopment of drug and test.
- Labeling Updates
- Biomarker Qualification Process

Biomarker Qualification Process

EVALUATION PROCESS
1) Informal discussion of a potential biomarker sponsor with the BQC
2) Biomarker Sponsor submits to BQC a written request for qualification of an exploratory biomarker.
3) BQC evaluates qualification request.
4) BQMT accepts or declines the sponsor's request to proceed with qualification process.
5) BQRT requests briefing document from biomarker sponsor.
6) BQ Project Manger schedules face-to-face meeting between the sponsor and the BQRT.
7) BQRT evaluates the briefing document and prepares for the Biomarker Qualification face-to-face meeting.
8) BQRT and Sponsor BQDS Meeting.
9) BQRT identifies and requests additional data from sponsor.
REVIEW PROCESS
10) BQRT receives full data package and review period begins
11) BQRT writes draft biomarker qualification review.
12) BQC routes the draft biomarker qualification reviews to all Offices
13) BQ Project Manager schedules the BQ review for presentation at a CDER Regulatory Briefing.
14) CDER Regulatory Briefing presentation and discussion is held.
15) CDER Office Directors make decisions to accept or reject the BQRT recommendations.
16) BQC drafts letter for sign-off by the Director of CDER communicating to the sponsor the results of the biomarker qualification..

DIA
Drug Information Association

Biomarker Qualification Context Claims

- **Nonclinical qualification completed**
  - Translational Biomarkers of Nephrotoxicity of the PSTC
- **Review Process**
  - Translational Biomarkers of Nephrotoxicity of ILSI/HESI
  - Galactomannan Test for Aspergillosis
  - Preclinical Application of Cardiac Troponins
- **Evaluation Process**
  - Toxicogenomic Analysis to Explore + Chromosome Ab
  - Circulating Tumor Cells in Metastatic Breast Cancer
  - Circulating Tumor Cells in Prostate Cancer
  - Translational Biomarkers of Hepatotoxicity of the PSTC

Is it a biomarker, or is it a test?

- Biomarkers are qualified with data supporting a specific context of use.
- Tests are cleared by CDRH with data supporting the analytical and clinical performance of the test.
- One biomarker could translate into many commercially available tests.

Biomarker Examples

- 1) eECG/ABBIOS is an electrocardiogram (ECG) computer analysis program, that allows fully automated 12-lead QT interval measurement, as well as manual adjudication
  - Fully automated mode supports:
    - Robust QT/QTc assessment in early clinical development
    - Thorough QT/QTc studies
- 2) Extraction of discrete 12-lead ECG strips from continuous Holter generates less noisy and more stable ECGs leading to more robust QTc data.