

Use of Transgenics in Carcinogenicity Testing

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Acknowledgements

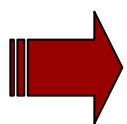
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- Joseph DeGeorge
- Chunhua Qin
- C-Path PSTC Carcinogenicity Working Group

Overview

- Development and evaluation of short-term carcinogenicity assays using transgenic mice – historical perspective
- Issues with utilization of transgenics in current test paradigms
 - selection of compounds with cause for concern
 - exposure, timing and resource issues
 - confidence in transgenic models for new classes
 - Ex. PPAR γ agonists and hemangiosarcoma in mice
- Understanding mode of action and human relevance of test results in transgenic mouse models
 - limitations of mouse models
 - confounding effect of species- and strain-specific susceptibility factors
 - applications of bioinformatics and omics technologies
- New directions for an integrated carcinogenicity testing strategy
 - genomic signatures for predicting carcinogenicity responses in the rat

Disadvantages and Limitations of the Lifetime Rodent Bioassay

- Time consuming (3+ years)
- Expensive (>\$1,000,000)
- Large number of animals required (~1000)
- Results do not contribute to mechanistic understanding
 - extensive follow-up studies required to determine mode of action
- Preponderance of species-specific responses
 - e.g. mouse liver tumors, rat bladder, kidney tumors, etc.
- Effects seen at maximum tolerated dose extrapolated to low levels of human exposure
- Human risk assessment problematic/growing list of “rodent” carcinogens



Need for effective alternative assays for human cancer hazard identification and risk assessment

Changing the Paradigm: A Step Forward

ICH Guideline (S1B) 1995

Testing for Carcinogenicity of Pharmaceuticals

The basic testing scheme should be comprised of:

- One long-term rodent carcinogenicity study
and
- One additional test for carcinogenic activity in vivo:
 - a short- or medium-term rodent test
 - initiation-promotion models
 - transgenic rodent models
 - neonatal rodent models
 - or
 - a long-term study in a second rodent species

Substitution allowed when data indicate alternative could provide additional information not likely to be obtained from traditional bioassay

Transgenic Models for Short-term Bioassays: In Pursuit of a Better Approach

- Leverage growing knowledge base of molecular mechanisms underlying human cancer
- Develop and evaluate in vivo bioassays with transgenic mouse models that
 - are engineered for accelerated tumorigenesis
 - by activation of oncogenes (ras)
 - inactivation of tumor suppressor genes (p53, XPA)
 - utilize genes of known importance in human cancer
 - have low spontaneous background incidence at study endpoint
 - are susceptible to chemically-induced acceleration of carcinogenesis
 - provide opportunities to understand mechanisms of carcinogenesis
 - respond more selectively to known human carcinogens
 - significantly reduce # of animals, time and costs involved in carcinogenicity testing

Progress in Development and Evaluation of Alternative Models

- Model development and characterization (1990 ->)
 - (MMTV-myc, -neu, -ras; Eu-pim, **rasH2**, **Tg.AC**, **p53**, **XPA**, p16, etc.)
 - Academic labs, NIEHS/NTP, CIEA, RIVM, Merck
- ICH S1B “Testing for Carcinogenicity of Pharmaceuticals” Guidance 1995 - 1997
- ILSI Alternatives to Carcinogenicity Testing Project 1996-2002
 - International cooperative scientific effort to evaluate alternative models
 - ILSI ACT workshops and publications
- Morton et al. (2002) The Tg rasH2 Mouse in Cancer Hazard Identification
- NIEHS/NTP: Role of transgenic mouse models in carcinogen identification (Pritchard, JB et al., 2003)
- Third IWGT Workshop and publication (2002/2004)
- ILSI ACT “Utility of Transgenic Assays for Risk Assessment” (2003/2004)
- RIVM Report (2004) on current status of alternatives

Current Transgenic Testing Models

- Tumor suppressor gene models for detection of genotoxic carcinogens
 - *p53*^{+/-}
 - *XPA*^{-/-}
 - *p53*^{+/-}/*XPA*^{-/-}
- ras oncogene models for detection of genotoxic and non-genotoxic carcinogens
 - *rasH2*
 - Tg.AC dermal (mechanistic relevance uncertain)
 - skin tumorigenesis = reporter phenotype for induced changes in gene expression by chemical or physical (wounding) agents

Models with the potential to inform with respect to mechanism

ILSI ACT Project: Academic Perspective*

A Role for Alternatives in Carcinogenicity Testing

- Models have potential utility as screening assays
 - < 100% accuracy of prediction of human carcinogenic hazard
- Use results in weight of evidence approach to risk assessment
- Models do have advantages
 - shorter duration, lower cost, fewer animals
 - less sensitive to non-genotoxic rodent carcinogens
 - more specific in predicting actual carcinogenic hazard to humans
- Limited usefulness for quantitative risk assessment & predicting target organ specificity for human carcinogenesis
- Specific models lack clear indication of mechanistic relevance
- Use in conjunction with data from other sources:
 - structure activity, genotoxicity, rat bioassay, other biological information

*Cohen, S (2001) Toxicol. Pathol. (Suppl 29)

ILSI ACT Project: Industry Perspective*

A Role for Alternatives in Carcinogenicity Testing

- Evaluation of accelerated carcinogenicity assays with transgenics should continue
- Greater clarity needed on their future role and required performance characteristics
- 5 areas for further discussion
 - standardize methods
 - optimal group size, study duration, statistical methods
 - chemicals/classes acceptable to find negative
 - integrate mechanism into evaluations
 - commonly owned compromises on changes to protocols
 - mechanism needed for adoption or rejection of assays

*Ashby, J. (2001) Toxicol. Pathol. (Suppl 29)

Technical Lessons Learned from ILSI Protocols for the Models

- Appropriateness of study durations
 - 26-weeks adequate for p53^{+/-}, rasH2, Tg.AC
 - XPA^{-/-} models require 9 months
- Appropriate use of positive controls
 - useful to include in study; otherwise confirm genotype
- Use wild-type mice in dose range-finding (XPA problematic)
- Criteria for a valid assay
 - MTD achieved in both sexes
 - positive control should be positive
- Use of non-tumor data in study evaluation
 - data from hyperplastic & pre-neoplastic lesions can support a positive result for rare tumors
- Use of embedded chip transponders not recommended

Technical Lessons Learned from ILSI Protocols for the Models

- Value of additional molecular endpoints
 - molecular analyses useful to characterize mechanism
 - LOH or mutation in wild type allele in induced tumors in p53^{+/-} informative
 - absence doesn't rule out a genotoxic mechanism (p-cresidine)
 - rasH2 (role for overexpression and mutation)
- Inclusion of high-dose, wild-type mice
 - can be valuable in understanding mechanism
 - is tumor response enhanced in transgenic vs wild type in p53 model
 - Yes: cyclophosphamide (genotoxic) diethylstilbestrol (genotoxic?)
 - No: cyclosporin (non-genotoxic)

Performance of Individual Models for Likely Human Carcinogens and Non-Carcinogens

| Strategy | Positive for Carcinogens | Negative for Non-Carcinogens | Positive for Non-Carcinogens | Negative for Carcinogens | Overall Accuracy |
|--|--------------------------|------------------------------|------------------------------|--------------------------|------------------|
| p53 ^{+/-} | 21 | 27 | 1 | 10 | 81% (48/59) |
| p53 ^{+/-} (GT) | 16 | 6 | 0 | 4 | 85% (22/26) |
| XPA ^{-/-} and/or XPA ^{-/-} /p53 ^{+/-} | 7 | 8 | 1 | 2 | 83% (15/18) |
| rasH2 | 21 | 18 | 5 | 7 | 76% (39/51) |
| Tg.AC | 23 | 17 | 18 | 0 | 69% (40/58) |

Carcinogens: ROC & IARC classification I, 2A, 2B (known, probable, possible)

Non-Carcinogens: IARC classification 3 (inadequate) or not evaluated, NTP(-)

*Pritchard et al., 2003; deVries, A., et al, RIVM Report 340700001, 2004.

Performance of Alternative (ICH) Strategy for Likely Human Carcinogens and Non-Carcinogens

| Strategy | Positive for Carcinogens | Negative for Non-Carcinogens | Positive for Non-Carcinogens | Negative for Carcinogens | Overall Accuracy |
|---------------------------------|--------------------------|------------------------------|------------------------------|--------------------------|------------------|
| NTP Rat Tg.AC (nGT) p53 (GT) | 35 | 13 | 9 | 0 | 84% (48/57) |
| NTP Rat rasH2 (nGT) p53 (GT) | 33 | 12 | 8 | 0 | 85% 45/53 |
| NTP Bioassays Rats and Mice | 23 | 17 | 18 | 0 | 69% 40/58 |

Carcinogens: IARC classification I, 2A, 2B

Non-Carcinogens: IARC classification 3

*Pritchard et al., 2003; deVries, A., et al, RIVM Report 340700001, 2004.

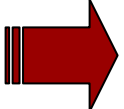
NIEHS/NTP Perspective & Strategy for Testing of Environmental Chemicals

- NTP proposed in 2002 to refine its testing paradigm to include genetically-altered mouse models (GAMMs)
- GAMMs have potential to:
 - to reduce time to complete an assay (2 yr -> 6-9 mo)
 - use fewer animals
 - provide greater mechanistic insight
- Most promising models:
 - p53^{+/-} for genotoxics/rasH2 irrespective of their genotoxicity
- Intention was to use GAMMs as an initial screen in most cases
- Proposed strategy on hold after Sept. 2002 NTP BSC meetings
- NTP had serious problems in acceptance of new methods
 - failure to identify agents that are known or potential human carcinogens
 - lack of agreement on value of positive and negative results in GAMMs
- NTP Vision
 - evolve from observational science with disease-driven models to predictive science based on target-specific, mechanism-based biological observations

Current Regulatory Status of the Models for Drug Safety Testing

- ICH guidelines and use of alternatives still supported
- rasH2 and p53^{+/-} most universally accepted models
 - US
 - p53^{+/-} likely to be preferred if evidence of genotoxicity
 - rasH2 may be allowed for genotoxic or non-genotoxic products
 - EU
 - likely to add value to carcinogenicity assessment
 - data do not suggest one model more appropriate for any compound class
- Tg.AC
 - useful only for dermally administered pharmaceuticals
 - issues
 - distinguishing promoters from compete carcinogens
 - dermal MTD and role of non-neoplastic effects (inflammation, irritation)
 - reacted inconsistently & incompletely to human carcinogens (EU/UK)
- XPA^{-/-} and XPA^{-/-} x p53^{+/-}
 - promising but more data needed; 9-month assay
 - XPA^{-/-} only relevant to bulky genotoxic carcinogens?

Transgenic Cancer Bioassays: A Useful Addition To Drug Safety Assessment?

- Scientific and regulatory consensus  yes
- Industry initially slow to adopt for routine use
 - smaller companies earliest adopters
 - significant cost savings
 - reluctance of large companies?
 - absence of large internal historical control databases for models
 - confidence in genetic toxicology testing paradigms
 - concern with potential for false positives with ras models
 - the “devil we know” perspective

The Case for Broader Utilization of Transgenic Models in Drug Safety Testing

- Patient benefits for earlier identification of compounds with carcinogenic potential
 - limit duration of human exposures in clinical trials (pos)
 - expand duration of human exposures in clinical trials (neg)
 - faster identification of viable back-ups when issues w lead seen
 - earlier access of improved compounds to patients with medical needs
- Early insight into mechanisms
 - ↓ responsiveness to indirect mechanisms of no human relevance
 - value of evidence for genotoxic/ non-genotoxic tumor mechanism
- Reduce the high costs of drug development
 - higher up-front animal costs but long-term manpower and resource savings
 - 26 wk vs 104 wk; 280 vs 500 mice
 - FTE costs - maintenance, dosing, necropsy, tissue proc., slide prep, path'y read
 - Material costs of drug, drug formulation, diet, facility overhead
 - early termination of a clinical program for a non-viable compound could save 10's of millions of dollars
 - earlier redirection to a viable development program could yield 100's of millions of marketing dollars
- Refinement and reduction of animal use

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 - Ex. PPAR γ agonists and hemangiosarcoma in mice

Selection of Compounds for Short-Term Alternative Bioassays

- Is there cause for concern for human relevant carcinogenic activity?
 - any ambiguous genotoxic potential
 - hormonal activity
 - immunosuppressive activity
- If yes
 - opportunity for rapid identification of compounds with potential carcinogenic effects in man
 - lower risk for indirect mechanisms that depend on (often species-specific) hormonal loops

Selection of Compounds for Short-Term Alternative Bioassays

- Is there cause for concern for carcinogenic activity of uncertain relevance to man
 - nuclear receptor agonist/antagonist activity (PPAR, RAR, new targets, etc.)
 - modulation of cell growth, differentiation, survival signaling pathways
 - earlier reads on positive findings for earlier investigations and follow-up action decisions

Selection of Compounds for Short-Term Alternative Bioassays

- Is there cause for concern for a signal of carcinogenic activity in rodents not relevant to man or likely to demonstrate a high threshold
 - evidence for non-genotoxic, rodent specific mechanisms
 - enzyme induction (liver)
 - cytotoxicity (liver, bladder, Kidney)
 - indirect hormonal mechanisms

Additional Points to Consider in Opting for a Short-Term Transgenic Model

Exposure Issues

- Transgenics are appropriate to use only when dose-limiting toxicity has been established
 - cannot rely upon saturation of exposure as high dose justification
 - when a 25X exposure justification is sought, prudent to use 2-year option rather than push to toxicity in transgenic
- Poorly absorbed compounds with GI pharmacology target
 - issue is testing GI exposure
 - 6 months dosing of rasH2 may be preferred model over 2-yr mouse getting 1500–5000 mpk

Additional Points to Consider in Opting for a Short-Term Transgenic Model

Timing and Resource Issues

- Unexpected delay after initiation of chronic rat studies
 - mouse rangefinding and 2-yr carco assay may be rate limiting
 - 6-month alternative may be preferred.
- Workload from several ongoing carco studies can stress path, tox, and facility resources
 - a flexible 6 month assay initiation would allow termination at a more convenient time well before filing deadlines.
- For lower priority compounds
 - can delay pre-investment in mouse rangefinding and Tg carco studies by at least 12 months
- Reduce drug costs ↓86%

Confidence in Transgenic Models for New Compounds & Class Effects

- Ex. Hemangiosarcoma as a class effect for PPAR γ agonists
- Utilization of transgenic assay as per ICH strategy disallowed pending further evaluation of model responses
 - ICH S1B criteria for alternative model selection
 - method should contribute to weight of evidence
 - need to provide scientific rationale for choice
 - » provide new information not likely to be obtained from 2-yr bioassay to inform hazard identification and risk assessment
 - » can model address concerns with prior knowledge of carcinogenic processes associated with a compound class?
- Recently published data shows rasH2 is weakly responsive to troglitazone-induced vascular tumors*
 - spleen hemangiosarcoma a common spontaneous tumor in rasH2

*Jin, M., et al., Arch Toxicol. (2007) 81:883-894

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Current Perspectives on Modeling Human Cancer in Mice

- Mouse models offer extraordinary tools for studying fundamental mechanisms of carcinogenesis
- But, many important species differences elucidated*
 - spectrum of common age-related neoplasms is very different
 - control of replicative senescence and ease of immortalization of murine cells reflect basic differences in telomere biology
 - key differences in signaling pathway perturbations required for cell transformation

Mouse cells

- ras (Raf-MAPK)
- p53

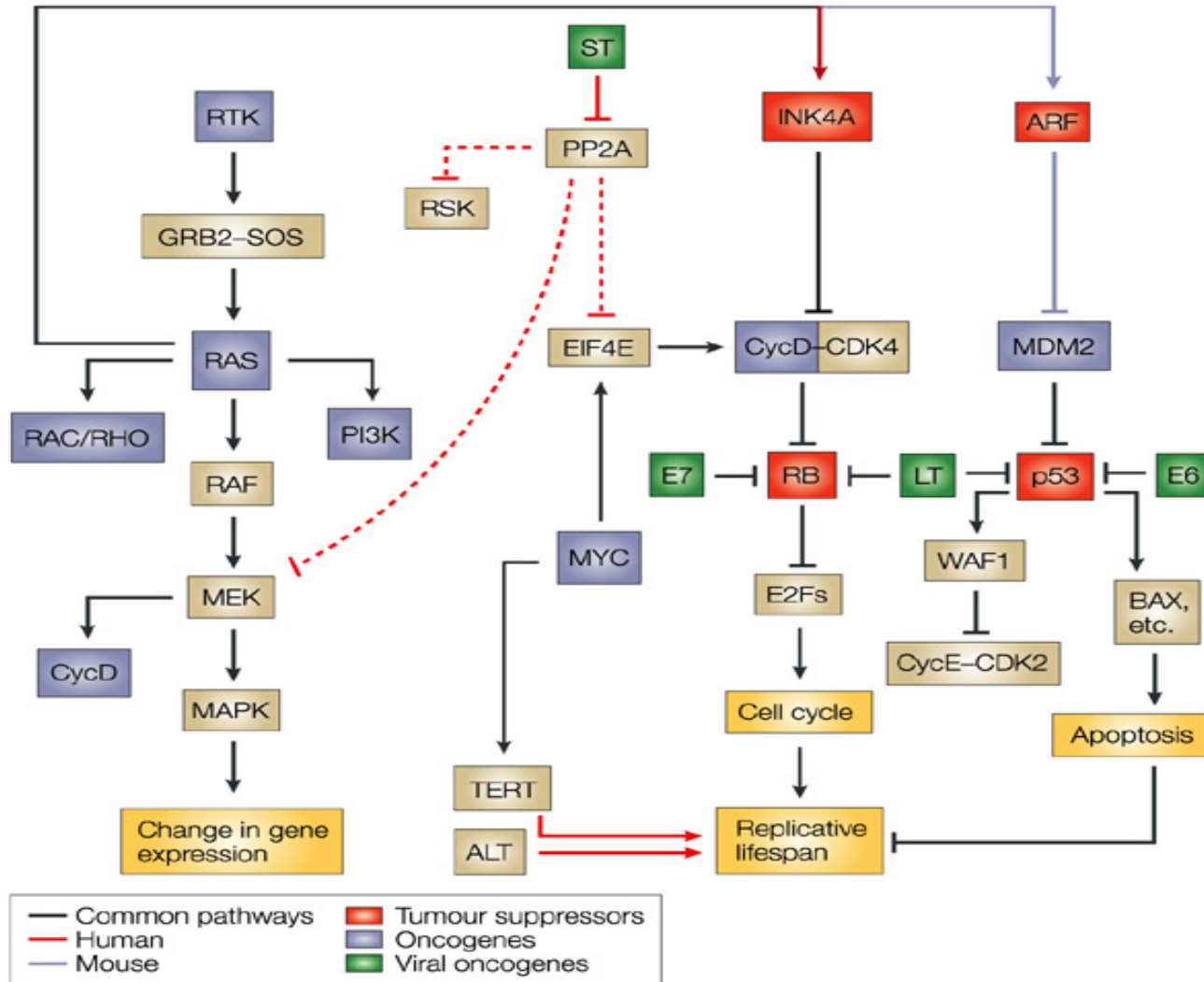
Human cells

- ras (Ral-GEF)
- p53
- Rb
- Telomerase
- Protein phosphatase 2A
- Additional ras effector pathways

- simpler processes of tumorigenesis in mice, fewer changes required?

* Rangarajan and Weinberg, Nature Reviews/Cancer 3:952-959, 2003

Modeling Human and Mouse Differences in the Molecular Circuitry of Cancer



Hahn, W.C. and Weinberg, R.A. (2002) Nature Reviews Cancer 2: 331-340

Humanized Mouse Models in Cancer Research

- Growing sophistication of current mouse models in cancer research
 - rapid progress with transgenic and endogenous GEMs in mimicking genetic drivers and kinetics of human cancer in mice
 - tissue- and disease-specific models
 - limiting cancer initiating populations
 - targeting cancer stem cells
- Further efforts to “humanize” mice
 - gene structure and regulatory elements
 - telomerase length and maintenance
 - drug metabolism
 - reconstituting the human immune system
 - manipulating the microenvironment

Potential Confounding Effects of Species- and Strain-Specific Susceptibility Factors

- Many responses in “humanized” mouse models likely to remain highly species- and strain-specific
 - “All models are wrong...some are useful” (George Box 1979)
- Integrated application of current technologies may allow us to identify species- and strain-specific susceptibility factors and assess human relevance
 - genomic/bioinformatic analysis of species and strain polymorphisms
 - SNPs, gene copy # variations and endogeneous retroviral elements
 - profiling genetic and epigenetically altered genes in tumors
 - systems biology approaches to identify common patterns of oncogene signaling collaborations
 - transcriptomic analysis of dysregulated genes in tumors
 - Ex. Troglitazone splenic hemangiosarcoma profile

Potential Confounding Effects of Species- and Strain-Specific Susceptibility Factors

- Ex. Differences in tumor susceptibility to urethane-induced lesions in rasH2 and p53
 - mediated by BALB/C vs C57BL/6 genetic backgrounds

| | Target Organ for Urethane Lesions | | |
|-----------------------|-----------------------------------|---------|--------|
| | Lung | Liver | Spleen |
| rasH2 | ++ (AdCa) | - | + (HO) |
| rasH2 WT | + (Ad) | - | - |
| BALB/c | + (Ad) | - | - |
| p53 ^{+/-} | - | ++ (HO) | - |
| p53 ^{+/+} WT | - | + (PH) | - |
| C57BL/6 | - | + (PH) | - |

High Level of Endogenous Retroviral Element Polymorphisms in Mouse Strains

PLoS GENETICS 4(2):1-14, 2008

Genome-Wide Assessments Reveal Extremely High Levels of Polymorphism of Two Active Families of Mouse Endogenous Retroviral Elements

Ying Zhang^{1,2}, Irina A. Maksakova^{1,2}, Liane Gagnier^{1,2}, Louie N. van de Lagemaat^{1,2*}, Dixie L. Mager^{1,2*}

¹Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, British Columbia, Canada, ²Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

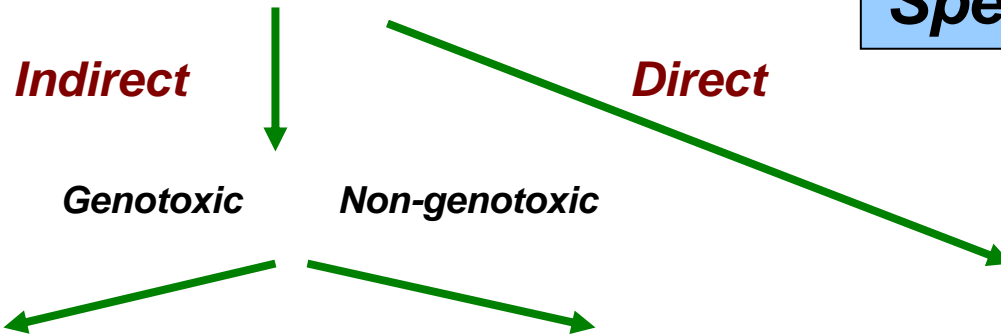
- IAP and ETn/MusD high-copy ERV elements are active retrotransposons (mutagens) in mice
- 60% of IAP and 25% of ETnMusD elements detected in any strain are absent in one or more of 3 other strains
- 700 polymorphic IAP, MusD ERV's or solitary LTRs found in gene introns
- Insertions associated with gene-splicing abnormalities and expression changes
- Comprehensive effort to document transposable element polymorphisms needed to complement SNP data and understand genotypic and phenotypic variation

Tg Mouse Tumorigenesis: Direct and Indirect Mechanisms of Toxicant Interaction with Host Susceptibility Factors



Chemical Mode of Action

Species- & Strain-Specific Susceptibility



- Endogenous Retroviruses (ERVs)
- SNPs/mutations
- Copy # variations

Toxicity and dysregulation of cell differentiation & proliferation balance in target tissue

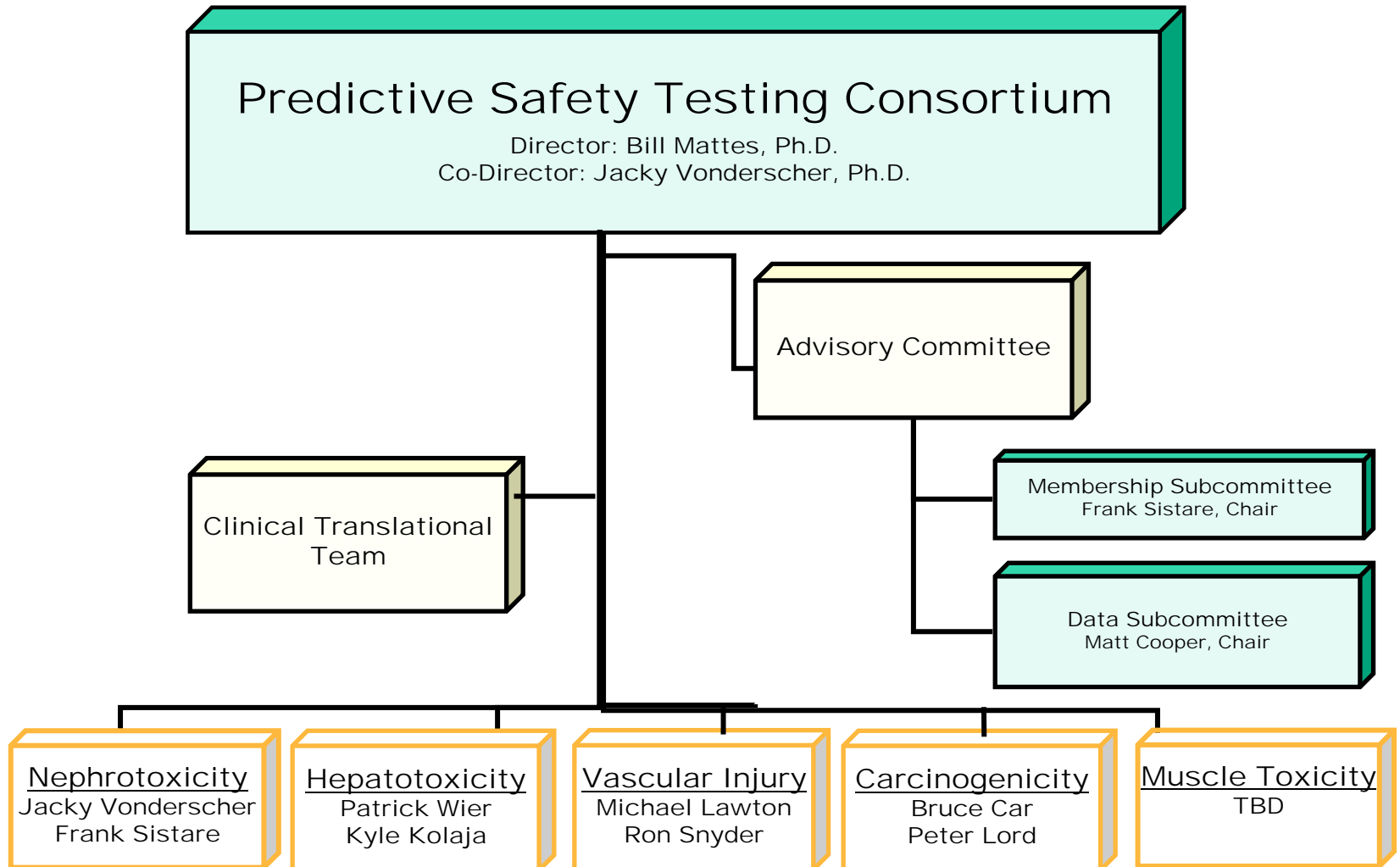
Induced gene expression & epigenetic Changes

Cell Transformation & Tumorigenesis

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 - **genomic signatures for predicting carcinogenicity responses in the rat**

C-Path PSTC Organization



“Inter-laboratory Evaluation of Genomic Signatures for Predicting Carcinogenicity in the Rat”

Predictive Safety Testing Consortium, Carcinogenicity Working Group, Mark R. Fielden, Alex Nie, Michael McMillian, et al, Toxicological Sciences 103(1): 28-34, 2008

Table 1. Comparison of Training Sets Used to Generate the Signatures

| Signature Evaluated | Training Set Size | Endpoint Predicted | Microarray Platform Used to Generate Signature | Rat Strain | Treatment Regime from Training Set |
|---|--|--|--|---------------------------|---|
| Iconix signature (Fielden et al., 2007) | 100 compounds (25 positive; 75 negative) | Non-genotoxic hepatocarcinogenicity | Codelink RU1 | 7-8 week old male SD rats | Samples collected 24h after 5 or 7 days of repeated daily administration at maximum tolerated dose ¹ |
| J&J signature (Nie et al., 2006) | 52 compounds (24 positive; 28 negative) | Non-genotoxic carcinogenicity (any tissue) | cDNA Microarray | 7-8 week old male SD rats | Samples collected 24hrs after single administration at maximum tolerated dose ² |

¹ For this test set, the maximum tolerated dose is defined as a dose intended to induce target organ toxicity or decreases in body weight gain after 5 days of daily dosing in the absence of significant clinical toxicity or body weight loss.

² The maximum tolerated dose was defined as approximately one half the published LD50, or higher if no or minimal gene changes were noted in the initial study.

Comparison of Test Sets Used to Evaluate Iconix and J&J Gene Signatures of Non-Genotoxic Carcinogenicity

Table 2. Comparison of Test Sets Used to Evaluate the Signatures

| Signature Evaluated | Test Set Site | Test Set Size | Microarray Platform Used to Evaluate Signature | Rat Strain | Treatment Regime from Test Set |
|--------------------------|---------------|---|--|---------------------------|--|
| Iconix 37 gene signature | J&J | 32 compounds (8 positive; 24 negative) | Codelink WG | 7-8 week old male SD rats | Samples collected 24hrs after single administration at maximum tolerated dose ¹ |
| Iconix 39 gene signature | GSK | 74 compounds (22 positive; 52 negative) | Affymetrix RAE230A | 7-8 week old male SD rats | Samples collected 24h after 4 days of repeated daily administration at maximum tolerated dose ² |
| J&J 6 gene signature | Iconix | 61 compounds (30 positive; 31 negative) | Affymetrix RG230v2 | 7-8 week old male SD rats | Samples collected 24h after single administration at maximum tolerated dose ² |
| J&J 6 gene signature | GSK | 78 compounds (25 positive; 53 negative) | Affymetrix RAE230A | 7-8 week old male SD rats | Samples collected 24h after 4 days of repeated daily administration at maximum tolerated dose ² |

¹ The maximum tolerated dose was defined as approximately one half the reported LD50, or higher if no or minimal gene changes were noted in the initial study.

² The maximum tolerated dose was defined as a dose intended to induce target organ toxicity or decreases in body weight gain after 4-5 days of daily dosing in the absence of significant clinical toxicity or body weight loss.

Evaluation of the Iconix Signature

Prediction

| | Pos. | Neg. | |
|------------------|------------|-----------|------------------------|
| Liver Carcinogen | 8 | 0 | Sensitivity: 100% |
| Non-Carcinogen | 9 | 15 | Specificity: 62.5% |
| | PP = 47.1% | NP = 100% | Accuracy: 71.9% |

J&J Test Set

| | Pos. | Neg. | |
|------------------|------------|------------|------------------------|
| Liver Carcinogen | 16 | 6 | Sensitivity: 72.7% |
| Non-Carcinogen | 21 | 31 | Specificity: 59.6% |
| | PP = 43.2% | NP = 83.8% | Accuracy: 63.5% |

GSK Test Set

P-value = 0.011

Evaluation of the J&J Signature

Prediction

| | Pos. | Neg. | |
|----------------|------------|------------|------------------------|
| Carcinogen | 16 | 14 | Sensitivity: 53.3% |
| Non-Carcinogen | 8 | 23 | Specificity: 74.2% |
| | PP = 66.7% | NP = 62.2% | Accuracy: 63.9% |

Iconix Test Set

| | Pos. | Neg. | |
|----------------|------------|----------|------------------------|
| Carcinogen | 24 | 1 | Sensitivity: 96% |
| Non-Carcinogen | 34 | 19 | Specificity: 35.9% |
| | PP = 41.3% | NP = 95% | Accuracy: 55.1% |

GSK Test Set

P-value = 0.002

Potential Next Steps with Tissue Molecular Biomarkers

- Generation of a joint CPath PSTC Taqman based PCR probe set to evaluate analytical fidelity across sample sets
- Reassess biological performance across all sample sets
- Signature refinement including input from alternate data sources
- Prospective test strategy
- Publication of results and discussion with Regulatory Authorities
- Depending upon outcome, discussion for integration with routinely conducted rat test study samples of X duration to assist negative predictivity

Summary

Utility of Transgenic Models in Carcinogenicity Testing Strategy

- Incorporating the 6-mo Tg mouse models in pharmaceutical testing strategies makes good scientific, ethical, and business sense
- Need integrated systems biology application of bioinformatics and omics technologies to better understand mode of action and human relevance of test results in Tg mouse models
 - assess potential roles of species and strain-specific susceptibility factors

New Directions for Integrated Carcinogenicity Testing Strategy

- Molecular tissue biomarkers being investigated by a PhRMA/FDA/EMA CPath-sponsored consortium
 - early mixed signs
 - some promise for assisting with negative prediction of rat tumor outcome

Acknowledgements

- Frank Sistare
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