Is the Two-Year Rodent Bioassay Needed to Address Carcinogenic Risk for Human Pharmaceuticals?

Frank D. Sistare, PhD

Safety Assessment and Laboratory Animal Resources

Merck & Co., Inc.

West Point, PA

frank_sistare@merck.com

Conflict of Interest Statement

- Frank Sistare is an employee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA

Outline

- ICH S1 Pharmaceutical Carcinogenicity Testing Guidance Modification Negotiations underway since 2012 proposes carcinogenicity assessments without the need for a 2-yr rat carco study
  - Where are we now and how did we get here? Case examples.

- New ICH S1 Guidance Sets the stage for a flexible Future Vision and creates demand and opportunity for a new tool box:
  - Rodent Carco studies for the near term will be more judiciously deployed. A box of new ***qualified tools*** will diminish their need over time.
  - New HESI Projects are launching to accelerate tool adoption.

Outline

- ICH S1 Pharmaceutical Carcinogenicity Testing Guidance Modification Negotiations underway since 2012 proposes carcinogenicity assessments without the need for a 2-yr rat carco study
  - Where are we now and how did we get here? Case examples.

- New ICH S1 Guidance Sets the stage for a flexible Future Vision and creates demand and opportunity for a new tool box:
  - Rodent Carco studies for the near term will be more judiciously deployed. A box of new ***qualified tools*** will diminish their need over time.
  - New HESI Projects are launching to accelerate tool adoption.

Current Pharmaceutical Carcinogenicity Testing Guidance

- S1A: GUIDELINE ON THE NEED FOR CARCINOGENICITY STUDIES OF PHARMACEUTICALS (1995)
  - any pharmaceutical used continuously for at least 6 months; used < 6 months but frequently/ intermittently for recurrent conditions; or if cause for concern (but < 6 months).
- S1B: TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS (1997)
  - basic testing scheme is one long-term rodent carcinogenicity study, plus one short or medium-term *in vivo* rodent test system (e.g., transgenic); or two long term rodent carcinogenicity studies.
- S1C(R2): DOSE SELECTION FOR CARCINOGENICITY STUDIES OF PHARMACEUTICALS (2008)
- S6(R1): PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS (2011)
  - rodent carc studies are of limited value; strategy is based on a weight of evidence approach; information can be sufficient to address carcinogenic potential without additional studies

2011 PhRMA Carcinogenicity Database Analysis Supports Modification of ICH S1 Guidelines

Toxicologic Pathology, 39: 716-744, 2011

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1177/0192623311406935

An Analysis of Pharmaceutical Experience with Decades of Rat Carcinogenicity Testing: Support for a Proposal to Modify Current Regulatory Guidelines

Frank D. Sistare[1], Daniel Morton[2], Carl Alden[3], Joel Christensen[1], Douglas Keller[4], Sandra De Jonghe[5], Richard D. Storer[1], M. Vijayaraj Reddy[1], Andrew Kraynak[1], Bruce Trela[6], Jean-Guy Bienvenu[7], Sivert Bjurström[8], Vanessa Bosmans[5], David Brewster[9], Karyn Colman[10], Mark Dominick[11], John Evans[8], James R. Hailey[12], Lewis Kinter[8*], Matt Liu[1], Charles Mahrt[13], Dirk Marien[5], James Myer[12], Richard Perry[2], Daniel Potenta[10], Arthur Roth[2], Philip Sherratt[1], Thomas Singer[9+], Rabih Slim[9], Keith Soper[1],

- Results of 190 pharmaceutical compounds and 76 IARC human carcinogenic chemicals = *266 total chemicals*
- NO histologic risk factors for neoplasia in a 6-month rat study + NO genetic toxicology + NO hormonal (or other pharmacologic) perturbation signals = NO value added from conducting a two-year rat carco study.
- 91% overall test sensitivity w no human relevant misses among the 14 false negatives in the 266 chemical database.
- The results of these analyses launched discussions to modify current ICH carcinogenicity testing guidelines, while maintaining patient safety, accelerating patient access, and projecting elimination of approx. 40% of two-year rat carco studies

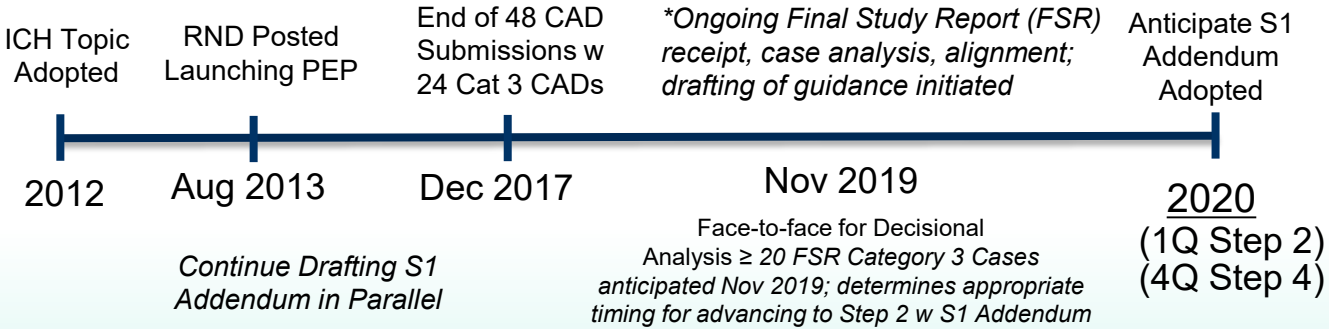
Starting With the End in Mind

In the absence of evidence of pharmacological (e.g., hormonal), toxicological (e.g., genotoxicity and histologic risk factors), and any mouse (transgenic) tumor response, the two-year rat bioassay provides little value in identifying potential carcinogenic risk.

In the presence of evidence of pharmacological, toxicological, or mouse findings suggesting potential carcinogenic risk, the two-year rat bioassay may provide information to clarify the level of risk.

Negotiations are in Progress to Modify Current ICH S1 Regulatory Guidance for Pharmaceutical Carcinogenicity Testing that Reduces Reliance on a Two-Year Rat Study

- **Regulatory Notice Document (RND) posted to ICH Website Aug 2013 launched Carcinogenicity Assessment Document (CAD) based Prospective Evaluation Period (PEP)**
  - PEP prospectively tests the assertion that a two-year rat bioassay is not always needed to inform carcinogenic risk:
    1) WOE criteria defined for a CAD from Sponsors to prospectively establish a carco outcome "prediction" with DRAs.
    2) Final two-year rat carcinogenicity study report will evaluate CAD prediction accuracy.



*S1 EWG Regulatory Members continue FSR evaluations

Weight of Evidence Elements for a Categorical Assignment in the CAD

1. Knowledge of intended drug target and pathway pharmacology, secondary pharmacology, & drug target distribution in rats and humans
2. Genetic Toxicology Study Results
3. Histopathologic Evaluation of Repeated Dose Rat Toxicology Studies with emphasis on Chronic Studies
4. Exposure Margins in Chronic Rat Toxicology Studies
5. Metabolic Profile
6. Evidence of Hormonal Perturbation
7. Immune Suppression
8. Special Studies and Endpoints
9. Results of Non-Rodent Chronic Study
10. Transgenic Mouse Study (not required for CAD prediction but can contribute if available)

2013 ICH S1 Regulatory Notice Document Launched a Prospective Verification for Sponsors to Assign a CAD Category Based on WOE

- **Category 1**: Highly Likely to be tumorigenic in humans Label as such. A two-year rat or two-year mouse or transgenic mouse study would not add value.
- **Category 2**: Tumorigenic potential for humans is uncertain. Rodent carco studies likely to add value to human risk assessment.
- **Category 3a**: Highly likely tumorigenic to rats but not humans from prior known mechanisms irrelevant to humans. A two-year rat study would not add value. Mouse study will suffice.
- **Category 3b**: High likely NOT to be tumorigenic to both rats and humans. A two-year rat study would not add value. Mouse study will suffice

NOTE THAT for Category 3a & 3b the RND proposes a mouse carco study be conducted even when no two-year rat study – likely that frequency of transgenic mouse deployment will grow

Current Status: 48 CADs were submitted, 24 of them being Category 3 (as defined by sponsor + at least 1 DRA)

EMERGING PROBLEM STATEMENT

- 48 CADs received: 34/48 CADs submitted by Sponsors as Category 3
  - **14** are unanimously Sponsor and DRAs (ALL) aligned
  - **10** are Sponsor and one or more DRAs aligned (DRA split decisions)
  - **10** are Sponsor Cat 3's BUT NO DRA agrees
- Of the other 14 CADs, both sponsor and DRA agree for 12 that a two-year rat study will add value
- 24 Final Study Reports (12 Category 3) reviewed by DRAs, learnings discussed w S1 EWG

What can be done to **MITIGATE DISCORDANCE, LEVERAGE LEARNINGS,** and enable a better future for carcinogenicity assessments that will not need a two-year rat study?

- 3 Inter-related Root-Cause Buckets of Discordance:
  1) Data in CADs viewed as insufficient; e.g., every tox study signal of risk is not adequately addressed
  2) First-in-class molecules, NEW TARGET/NEW BIOLOGY/NEW CHEMISTRY viewed w higher uncertainty
  3) Demonstrating a negative is viewed as too valuable to pass up (so need to deliver more than a negative 6 mo. transgenic, negative w-o-e argument to sufficiently convince all regulatory authorities)

What About Category 2? Still Expecting Value from Conducting a "Definitive" Two-Year Rat Carco Study

Case 1: **First-in-class** for a serious but not a debilitating/ life threatening disease

- Literature review indicates **significant potential for a plausible tumorigenic target based mechanism**
- Negative for endocrine, gene tox, chronic study histologic findings

Case 2: **First-in-class** for prevention of a debilitating degenerative disease

- Literature review indicates minimal potential for a plausible tumorigenic target based mechanism
- **Hepatocellular hypertrophy reported without mechanistic explanation**

Case 3: **First-in-class** for a viral infectious disease

- Antiviral target; so no on-target mechanistic concerns
- Negative for endocrine, histo; BUT ***in vivo*** **gene tox at high exposures; will tumors be seen in two-year at lower margins?**

Non-Starter Case (Not in any Category):

- First-in-class for serious but not life threatening disease
- Literature review indicates **significant potential for a plausible target based human relevant tumorigenic mechanism, cooperating w a driver gene pathway**
- Conditional KO mouse constructed, **shows tumors.** **Drug halted. Tumors expected in rodents and humans.**

Evidentiary Criteria Considerations for First-in-Class Waiver Proposals

1) How critical would the impact of a false-negative be on human health risk?

Impact on Human Health Risk

Less impact of a potential wrong waiver decision	Higher impact
Use limited to elderly	Pediatric/ broad target population
Debilitating Disease Indication	Non-debilitating Disease
No satisfactory alternative therapeutics	Therapeutic alternatives available

2) How valuable would a two-year study be; would an alternative approach be superior?

Value of Two-Year Rat Study Data vs. Alternative Approach

Less Value of a two-year rat study	Higher value
Rat provides poor pharmacologic target coverage	Target affinity, signaling pathway, tissue distribution matches human
Rat exposure is very limited and metabolite coverage is deficient	Good parent and metabolite exposure coverage
Rat known to be poor model of human risk (e.g., immunosuppression)	No alternate approach is known to be more meaningful

3) How convincing are all available data on *the novel target*?

Understanding of Novel Drug Target Hypothetical Risks

Drug Target Convincingly Safe	Theoretically Reasonable Safety Concerns
Animal GEMs of target well characterized as safe	Human genetics data indicate target is cancer driver gene
Comprehensive literature search indicates low risk	Numerous investigators recapitulate relevant cancer risk.
Comforting experience w other "similar type" drug targets & pathways	Other "similar type" drug targets have raised concerns
Tissue distribution is known to be limited	Broad tissue distribution/endocrine organ tissue distribution
Closely related target protein subtypes similarly characterized as safe	Concerns from closely related target protein interactions

4) How convincing and complete are all of the *specific drug study data* from the sponsor?

Comprehensive Study Data Package

Less Value of a two-year rat study	Higher value
No NEGCARC signals in all studies and high exposures achieved	Convincing NEGCARC signals of concern
Or minimal NEGCARC signals only at highest exposures, and all fully explained	NEGCARC signals not fully explained
Theoretical concerns based on target pharmacology addressed creatively	Structural class concerns (e.g., nucleosides)
Data package indicates high target specificity, low off-target potential	Evidence for high drug promiscuity, poor specificity
Well characterized / behaved ADME	Evidence for slow tissue accumulation, dynamic ADME over time

Outline

- ICH S1 Pharmaceutical Carcinogenicity Testing Guidance Modification Negotiations underway since 2012 proposes carcinogenicity assessments without the need for a two-year rat carco study
  - Where are we now and how did we get here? Case examples.

- New ICH S1 Guidance Sets the stage for a flexible Future Vision and creates demand and opportunity for a new tool box:
  - Rodent carco studies for the near term will be more judiciously deployed. A box of new ***qualified tools*** will diminish their need over time.
  - New HESI Projects are launching to accelerate tool adoption.

Two Key Problems Will Limit Implementation of Anticipated S1B Guidance and Present Two Important Opportunities for HESI Projects to Maximize Its Benefits

PROBLEM #1: Approximately 50% of pharmaceuticals in six-month rat studies present w "histologic risk factors of neoplasia". ***These require explanation for a two-year rat carc study waiver.***

SOLUTION: ***Use transcriptomic signatures to identify common molecular initiating mechanisms.***

- By far, the most common organ presenting with histologic risk factors is liver. Certain mechanisms underlying such "histologic risk factors of neoplasia" in liver are associated w liver tumors and/or tumors in other tissues (e.g., testes, thyroid, etc.) thru indirect mechanisms considered human irrelevant. Three common mechanisms:
  a) **CAR mediated liver enzyme induction,**
  b) **PXR mediated liver enzyme induction,**
  c) **PPARα mediated liver enzyme induction**.
- On the other hand, tumorigenic mechanisms can be human relevant, requiring careful analyses before further investment. It is important to know if a drug candidate exhibiting "histologic risk factors of neoplasia," may also exhibit human relevant tumorigenic liability, and this needs to be ruled out. These mechanisms can include:
  a) **Reactive hyperplasia following repeat tissue injury**
  b) **DNA damaging genotoxicity**
  c) **Sustained and prolific AhR receptor activation**
  d) **Sustained estrogen receptor activation**

Two Key Problems Will Limit Practical Implementation of the Anticipated S1B Guidance and Present Two Important Opportunities for HESI Projects to Maximize Its Benefits

PROBLEM #2: "First-in-class" pharmaceuticals are receiving lower levels of alignment between DRAs, and Sponsors. DRAs are generally cautious and reluctant to issue study waivers for first-in-class molecules even ***when there are NO histologic risk factors of neoplasia in all tox studies. Negative 2-yr rat studies are viewed w higher value.***

SOLUTION: *follow the logic of biology*. Additional approaches and analyses of samples from "clean studies" may be needed to enhance regulatory confidence that first-in-class molecules can qualify for a waiver. Experts have asserted that limited modes of action exist for carcinogenesis that can be generally bucketed into 3 categories: cell survival, genome maintenance, and cell fate. ***Can error-corrected NGS together with novel transcriptomic signatures always inform when a drug may or may not drive growth advantaged sub-clonal mutations associated with cell survival, cell fate or genome maintenance.***

a) What unique compounds, study samples and existing metadata can pharma share toward this purpose?
b) Is it reasonable to anticipate that new opportunities will emerge for new transcriptomic signatures and other new molecular endpoints to be leveraged?
c) If so, then opportunity will present to qualify such approaches when better understood, thoroughly evaluated, and together we can grow confidence.

Two Key Problems Will Limit Implementation of Anticipated S1B Guidance and Present Two Important Opportunities for HESI Projects to Maximize Its Benefits

PROBLEM #1: Approximately 50% of pharmaceuticals in six-month rat studies present w "histologic risk factors of neoplasia". ***These require explanation for a two-year rat carc study waiver.***

SOLUTION: ***Use transcriptomic signatures to identify common molecular initiating mechanisms.***

- By far, the most common organ presenting with histologic risk factors is liver. Certain mechanisms underlying such "histologic risk factors of neoplasia" in liver are associated w liver tumors and/or tumors in other tissues (e.g., testes, thyroid, etc.) thru indirect mechanisms considered human irrelevant. Three common mechanisms:
  a) **CAR mediated liver enzyme induction,**
  b) **PXR mediated liver enzyme induction,**
  c) **PPARα mediated liver enzyme induction**.
- On the other hand, tumorigenic mechanisms can be human relevant, requiring careful analyses before further investment. It is important to know if a drug candidate exhibiting "histologic risk factors of neoplasia," may also exhibit human relevant tumorigenic liability, and this needs to be ruled out. These mechanisms can include:
  a) **Reactive hyperplasia following repeat tissue injury**
  b) **DNA damaging genotoxicity**
  c) **Sustained and prolific AhR receptor activation**
  d) **Sustained estrogen receptor activation**

Posted OECD (Organisation for Economic Co-operation and Development) Adverse Outcome Pathway (AOP) Targets of Interest Align Well HERE:

August 2017 Work Plan for the Test Guidelines Program:

- Projects 4.94, 4.125 dealing with aspects of Non-Genotoxic Carcinogenicity
- Project 2.57, 2.61, 4.73, 4.99, all dealing with aspects of androgen receptor activation
- Project 4.101, 4.119 dealing with aspects of estrogen receptor activation

AOP-Wiki release 2.2 (Jan 28, 2018) most listed as “under development”

- ID 111, 117, 120 (Alteration of Androgen R & rodent carcinogenesis)
- ID 53, 165, 199, 200 (Estrogen R and rodent tumors)
- ID 60, 107, 162, 220 (Enzyme induction/ CAR/ PXR/ T3/4 clearance & Rodent Tumors)
- ID 109, 116, 118 (cytotoxicity and rodent tumorigenesis)
- ID 166, 37 (PPARa)
- ID 41 (sustained AhR activation) [open for citation & comment]

Liver ADME Gene Expression Scores from >400 Early Rat High Dose Tolerability Studies at Merck Show that Potentially Confounding Nuclear Receptor Activation is VERY Common

- **AhR Gene Signatures**
  - Overall **4%** of the compounds tested, presented with a Significantly Positive Score (seen in 3 programs)
  - An additional **20%** of compounds tested, presented with weaker positive AhR Scores of less concern

- **CAR Gene Signatures**
  - Overall **20%** of compounds tested had positive CAR dominant signatures

- **PXR Gene Signatures**
  - Overall **30%** of compounds tested had positive PXR dominant signatures

- **PPARα Gene Signatures**
  - Overall **16%** of compounds tested had positive PPARα signatures

NOTE: these are initial HIGH DOSE tolerability studies, so doses may not be appropriate for two year rat carc studies

First-in-Class Molecule Case Example w a Human Relevant Off-Target Risk Factor: AhR Activation

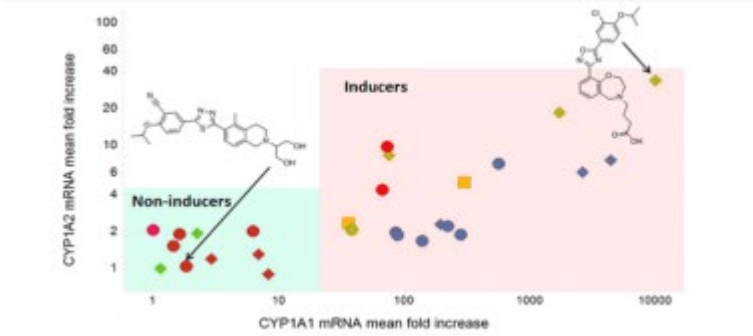
Example: De-Risking *Reactively* for AhR Agonism Carcinogenicity Concern Seen in Drug Discovery at GSK

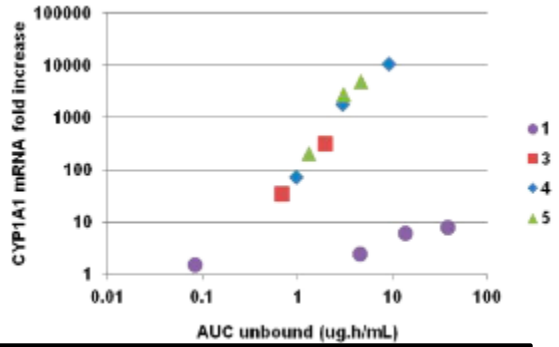
Navigating CYP1A Induction and Arylhydrocarbon Receptor Agonism in Drug Discovery. A Case History with $S1P_1$ Agonists

Simon J. Taylor*†, Emmanuel H. Demont†, James Gray†, Nigel Deeks†, Aarti Patel‡, Dung Nguyen§, Maxine Taylor‡, Steve Hood‡, Robert J. Watson†, Rino A. Bit†, Fiona McClure∥, Holly Ashall∥, and Jason Witherington†

† Immuno-Inflammation Therapy Area Unit, GlaxoSmithKline, Gunnels Wood Road, Stevenage, SG1 2NY, U.K.
‡ PTS DMPK, GlaxoSmithKline, Park Road, Ware, SG12 0DP, U.K.
§ PTS DMPK, GlaxoSmithKline, Upper Merion, 709 Swedeland Road, King of Prussia, Pennsylvania 19406, United States
∥ Safety Assessment, GlaxoSmithKline, Park Road, Ware, SG12 0DP, U.K.

J. Med. Chem., **2015**, *58* (20), pp 8236–8256





This article describes the finding of substantial upregulation of mRNA and enzymes of the cytochrome P450 1A family **during a lead optimization campaign for small molecule $S1P_1$ agonists. Fold changes in mRNA up to 10 000-fold for CYP1A1 in vivo in rat and cynomolgus monkey** and up to 45-fold for CYP1A1 and CYP1A2 *in vitro* in rat and human hepatocytes were observed. Challenges observed with correlating induction *in vitro* and induction *in vivo* resulted in **the implementation of a short, 4 day *in vivo* screening study in the rat which successfully identified noninducers**.

2007 Iconix Publication: AhR Agonists That are Non-persistent are Not of Toxicologic Concern

Induction of Cyp1a1 Is a Nonspecific Biomarker of Aryl Hydrocarbon Receptor Activation: Results of Large Scale Screening of Pharmaceuticals and Toxicants in Vivo and in Vitro

Wenyue Hu, Claudio Sorrentino, Michael S. Denison, Kyle Kolaja, and Mark R. Fielden

Molecular Pharmacology June 2007, 71 (6) 1475-1486; DOI: http://dx.doi.org/10.1124/mol.106.032748

"…To evaluate the accuracy of *in vivo* Cyp1a1 induction as a biomarker of AhR agonist activity, we evaluated rat gene expression data in DrugMatrix, a large toxicogenomic database of gene expression profiles for 596 compounds (Ganter et al., 2005), and found that Cyp1a1 was induced by 239 compounds in a variety of tissues. The majority of the active compounds are marketed drugs with toxicity profiles unlike those produced by exposure to HAHs. To evaluate the sensitivity and specificity of *in vivo* Cyp1a1 induction to identify AhR agonists, a subset of 147 compounds was evaluated using a combination of *in vitro* assays to assess their ability to stimulate AhR transformation and DNA binding, dioxin response element (DRE)-driven reporter gene expression, and to compete with dioxin for binding to the AhR. The *in vivo* expression of other AhR-regulated genes, including Cyp1a2, Ugt1a1, and Nqo1, was also evaluated to determine whether the expression of these DRE-driven genes could improve the accuracy for identifying AhR agonists. Although all AhR agonists induce Cyp1a1 gene expression, the induction of Cyp1a1 expression *in vivo* does not necessarily implicate that a chemical is a direct AhR agonist. Furthermore, six marketed drugs that activate and bind to the rat AhR were identified and many treatments that induce Cyp1a1 in a tissue-specific manner and in a distinct pattern relative to other AhR-regulated genes. **These results lend support to the hypothesis that AhR activation is not synonymous with AhR agonist activity and HAH-like toxicity for nonpersistent compounds..."**

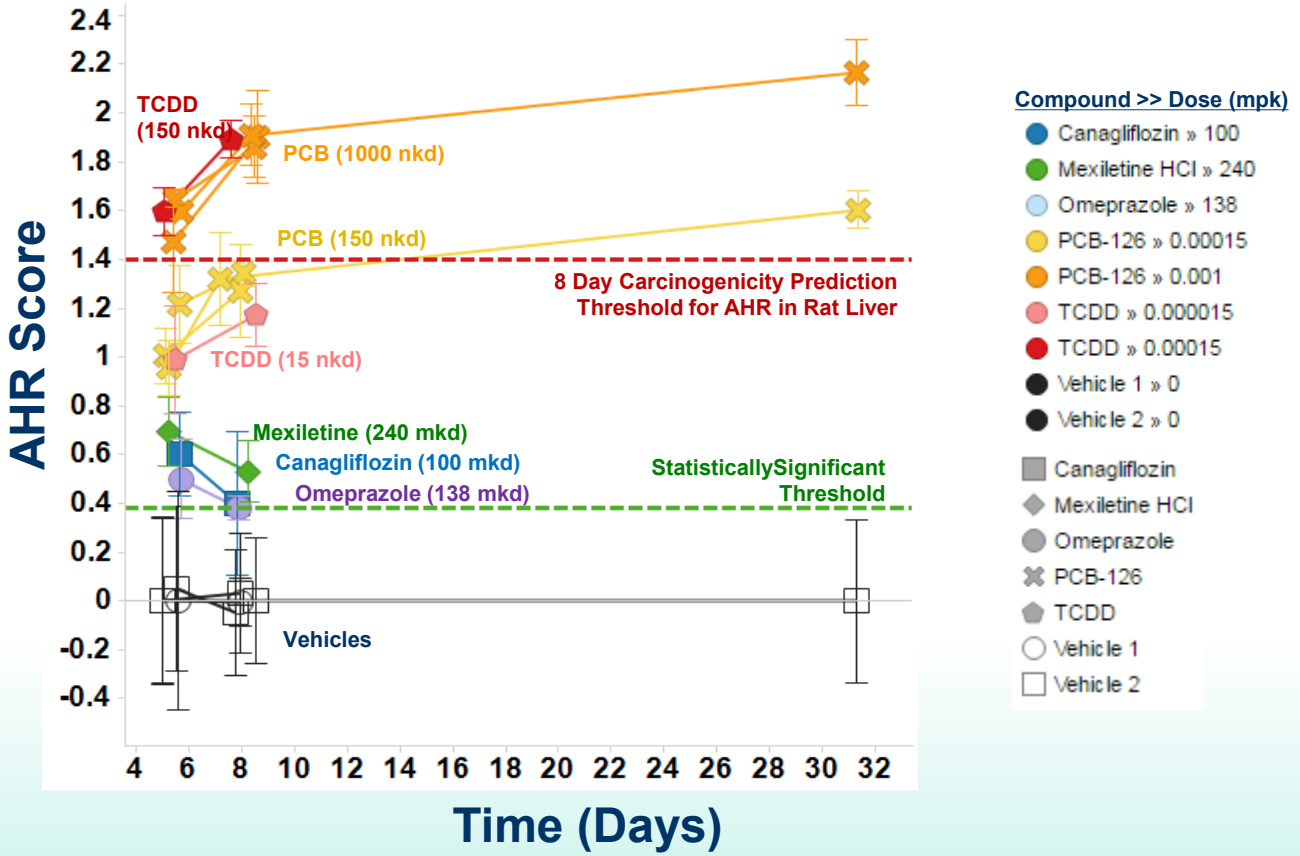
With respect to estimates of dioxin-like toxicity, a rich body of literature indicates that metabolically persistent halogenated ligands of the AhR cause sustained activation of the receptor and result in a wide spectrum of toxic responses similar to TCDD, whereas metabolically labile, nonhalogenated AhR ligands do not typically produce dioxin-like toxicities in animal studies. ….These results suggest that whereas binding and activation of the AhR are necessary prerequisite events for AhR-dependent dioxin-like toxicity, the actual occurrence of toxicity requires both continual presence of the AhR agonist and persistent activation of the AhR signaling pathway. In the current study, through a combination of *in vivo* and *in vitro* assays, a number of weak AhR ligands were identified, including nimodipine, leflunomide, flutamide, omeprazole, mexiletine, and atorvastatin. These compounds, which are approved for use by the U.S. FDA, do not produce dioxin-like toxicities in rats, and there is no evidence for chloracne, immunosuppression, or other adverse dioxin-like effects in exposed humans. This could be due to both their reduced potency relative to TCDD and/or their rapid rate of clearance from the body relative to persistent halogenated ligands. It would seem that **the toxicological consequences of transient or weak receptor activation are qualitatively and quantitatively distinct from persistent activation by metabolically stable and potent ligands.**

Hypothesis for AHR Association with Tumorigenesis

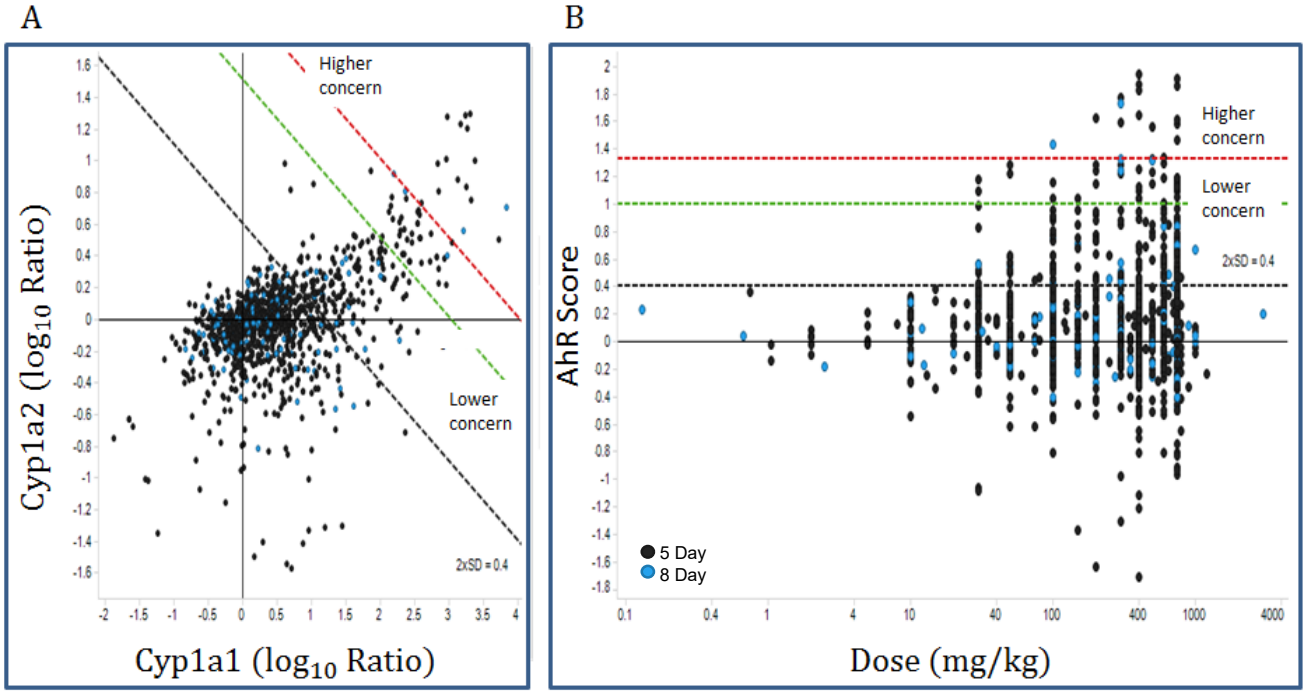
Threshold Magnitude Crossed	Sustained Activation

The Eventual Rat Carco Study Dose Must Be Considered for Proper Perspective

TCDD and PCB126 Discriminate the Same Tumorigenic Thresholds and Sustain AhR Activation. Two-year Rat Study Doses of Omeprazole, Mexiletine, and Canagliflozin Fall Well Below Threshold and Do Not Sustain.



Internal Merck Experience with (A) Cyp1a1 and Cyp1a2 Induction, and (B) AhR Scores Across >700 Drug Candidates Considered for Early Drug Development



Two Key Problems Will Limit Practical Implementation of the Anticipated S1B Guidance and Present Two Important Opportunities for HESI Projects to Maximize Its Benefits

PROBLEM #2: "First-in-class" pharmaceuticals are receiving lower levels of alignment between DRAs, and Sponsors. DRAs are generally cautious and reluctant to issue study waivers for first-in-class molecules even ***when there are NO histologic risk factors of neoplasia in all tox studies. Negative two-year rat studies are viewed w higher value.***

SOLUTION: *follow the logic of biology*. Additional approaches and analyses of samples from "clean studies" may be needed to enhance regulatory confidence that first-in-class molecules can qualify for a waiver. Experts have asserted that limited modes of action exist for carcinogenesis that can be generally bucketed into 3 categories: cell survival, genome maintenance, and cell fate. ***Can error-corrected NGS together with novel transcriptomic signatures always inform when a drug may or may not drive growth advantaged sub-clonal mutations associated with cell survival, cell fate or genome maintenance.***

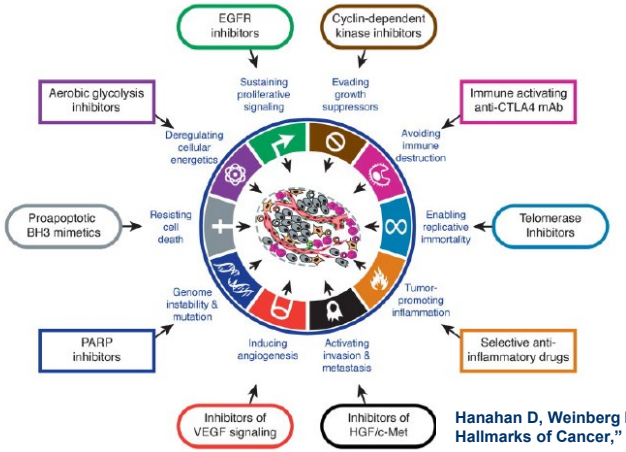
a) What unique compounds, study samples and existing metadata can pharma share toward this purpose?
b) Is it reasonable to anticipate that new opportunities will emerge for new transcriptomic signatures and other new molecular endpoints to be leveraged?
c) If so, then opportunity will present to qualify such approaches when better understood, thoroughly evaluated, and together we can grow confidence.

Leveraging Comprehensive Human DNA Sequencing Data to Inform Target-Related Potential for Pharmaceutical Carcinogenicity Risk*

Human Tumor Sequencing Data and Hereditary Germline Cancers Revelations:

1) 500+/- Driver Genes confer selective growth advantages by regulating 3 core processes
   a) Cell survival (e.g., ras pathway, P53, PI3K)
   b) Genome maintenance (DNA damage repair pathways)
   c) Cell fate (e.g., NOTCH, APC)

"Discovery of the molecular components of these pathways is one of the greatest achievements of biomedical research." *



*B. Vogelstein, et al (2013) "Cancer Genome Landscapes," Science 339: 1546-1558

Hanahan D, Weinberg RA, (2000) "The Hallmarks of Cancer," Cell 100: 57-70

Leveraging Comprehensive Human DNA Sequencing Data to Inform Target-Related Potential for Pharmaceutical Carcinogenicity Risk*

I. Human Tumor Sequencing Data Revelations:

1) 500 +/- genes are altered (mutations/deletions/translocations/ amplifications, etc.) in a high percentage of tumors and so, are considered "driver genes" as they confer a selective growth advantage
2) Many more "passenger gene" mutations altered infrequently that confer no growth advantage
3) Typical tumor contains 2–8 driver gene mutations
4) Certain tumor types display many more total mutations (≥200/tumor) than average (10 –100/tumor)
   - Melanomas (*UV light*)
   - Lung tumors from *smokers* (≥200/tumor) vs non-smokers (10-20/tumor)
   - Colorectal (*defective DNA repair*) (500-1500/tumor)

*B. Vogelstein, et al (2013) "Cancer Genome Landscapes," Science 339: 1546-1558

Leveraging Comprehensive Human DNA Sequencing Data to Inform Target-Related Potential for Pharmaceutical Carcinogenicity Risk*

II. Human Tumor Sequencing Data Revelations:

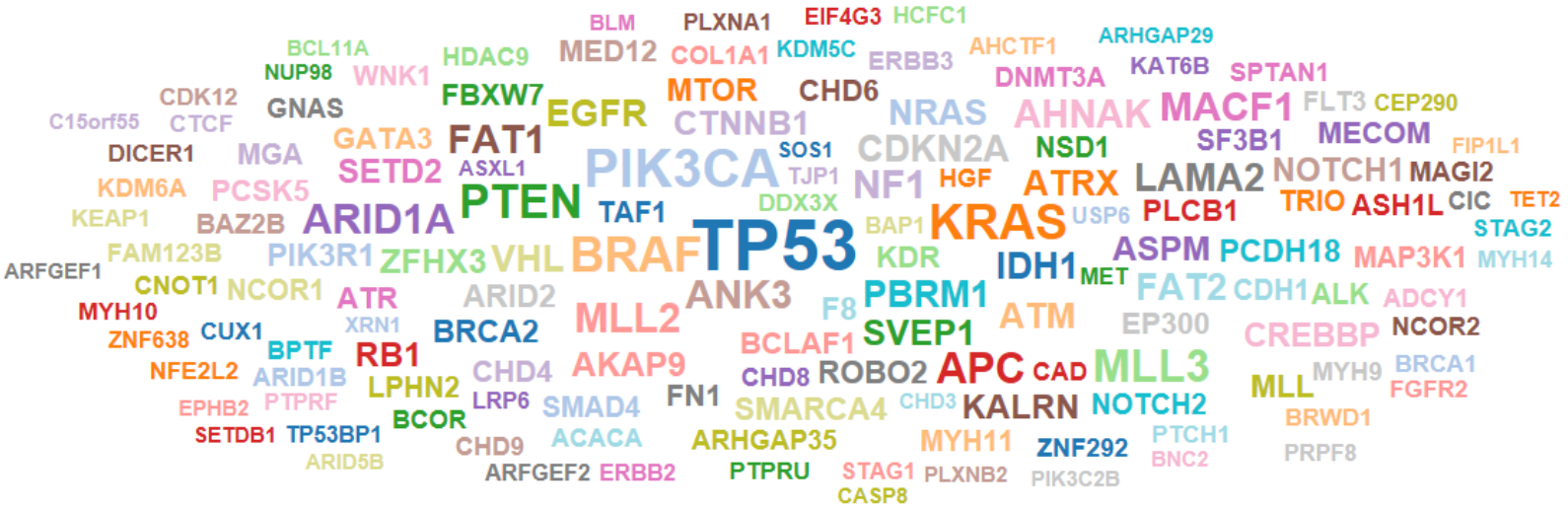
1) Numerous statistical methods have been applied to identify driver genes from passenger gene alterations
   a) Genes with high frequency of mutations indicate likely a driver gene (likely that a causal association exists)

2) COSMIC (Catalogue of Somatic Mutations in Cancer)
   a) >18,000 mutated genes across >3000 tumors. 138 concluded to be driver genes.
   b) 64 Oncogenes=activating, and >20% of recorded mutants are recurring
   c) 74 Tumor Suppressor Genes=inactivating, and >20% recurring

*B. Vogelstein, et al (2013) "Cancer Genome Landscapes," Science 339: 1546-1558

Human Cancer Driver Genes

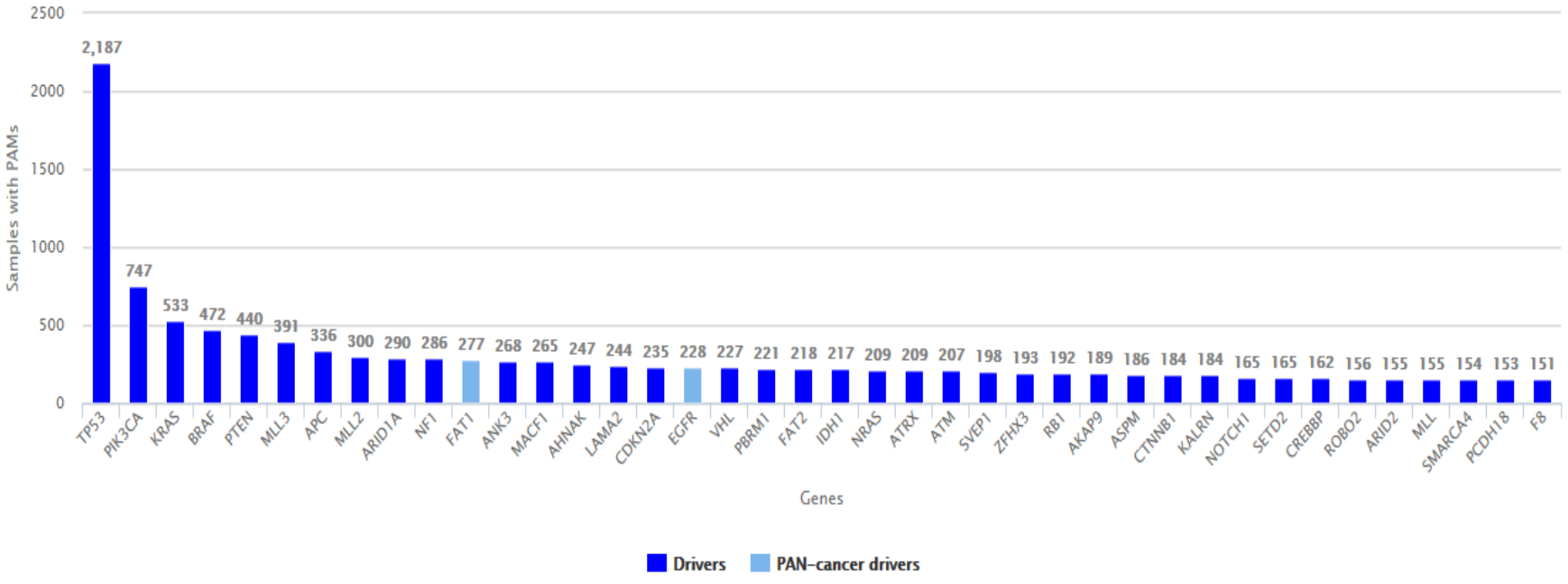
Mutational cancer driver genes: 459



This driver cloud represents the most recurrently mutated **cancer driver genes**. The size of the gene symbol is relative to the count of samples with PAMs.

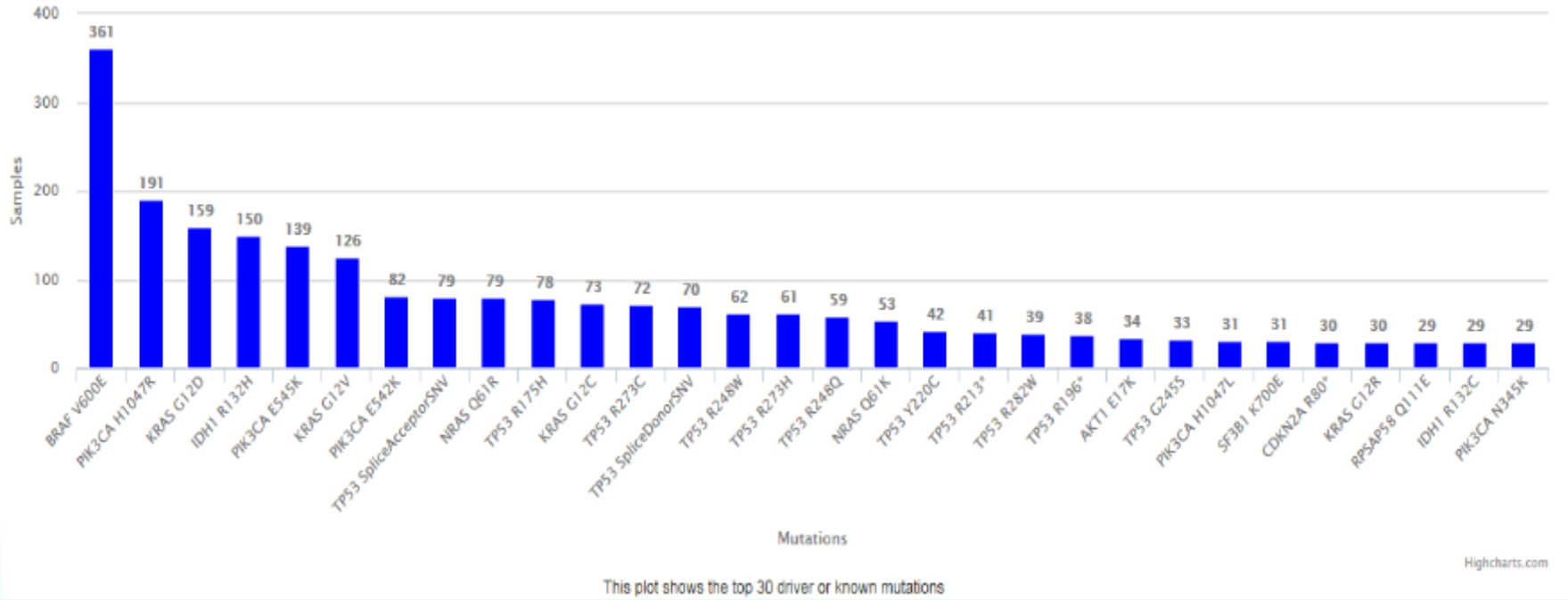
https://www.intogen.org/search?

Catalog of Human Mutated Cancer Driver Genes: Frequency Distribution (Top 40)



This plot shows the most recurrently mutated **cancer driver genes**. Each bar of the histogram indicates the amount of samples with PAMs.

Catalog of Human Mutated Cancer Driver Genes: Frequency Distribution of Top 30 Specific Mutations



This plot shows the top 30 driver or known mutations

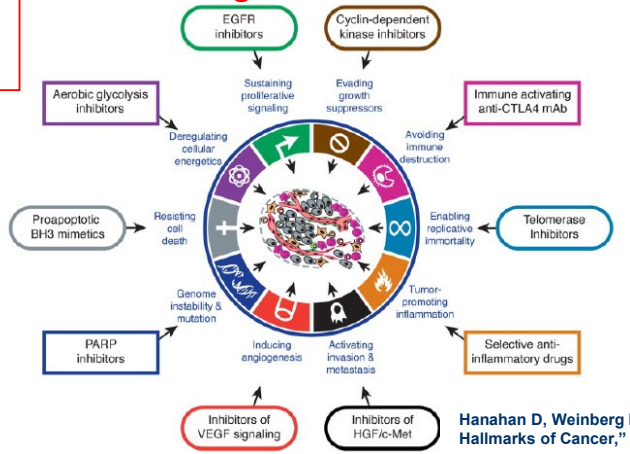
Leveraging Comprehensive Human DNA Sequencing Data to Inform Target-Related Potential for Pharmaceutical Carcinogenicity Risk*

Human Tum… Cancers Revelations:

1) 500+/-… es by regulating 3 core processes
   a) Cell s…
   b) Geno…
   c) Cell f…

"Discovery … one of the greatest achievements of biomedical …

ALL TUMORIGENS Will CAUSE, ENCOURAGE, ALLOW…. the Accumulation of Driver Gene Mutations that confer a Selective Clonal Growth Advantage



*B. Vogelstein, et al (2013) "Cancer Genome Landscapes," Science 339: 1546-1558

Hanahan D, Weinberg RA, (2000) "The Hallmarks of Cancer," Cell 100: 57-70

Ultra-sensitive sequencing for cancer detection reveals progressive clonal selection in normal tissue over a century of human lifespan

Jesse J. Salk[1,2], Kaitlyn Loubet-Senear[3†], Elisabeth Maritschnegg[4††], Lindsey N. Williams[2], Reinhard Horvat[5], Adriaan Vanderstichele[6], Kathryn T. Baker[3], Mary J. Emond[7], Emily Loter[8], Thierry Soussi[9,10,11], Robert Zeillinger[4], Paul Speiser[4] and Rosa Ana Risques[3*]

bioRxiv preprint first posted online Nov. 4, 2018;
doi: http://dx.doi.org/10.1101/457291.

Molecular Diagnosis & Therapy (2018) 22:603–611
https://doi.org/10.1007/s40291-018-0350-z

ORIGINAL RESEARCH ARTICLE

Validation Strategy for Ultrasensitive Mutation Detection

Marija Debeljak[1] · Michael Noë[1,2] · Stacy L. Riel[1] · Lisa M. Haley[1] · Alexis L. Norris[1] · Derek A. Anderson[1] · Emily M. Adams[1] · Masaya Suenaga[1] · Katie F. Beierl[1] · Ming-Tseh Lin[1] · Michael G. Goggins[1,2,3,4] · Christopher D. Gocke[1,2] · James R. Eshleman[1,2,4]

Published online: 5 July 2018

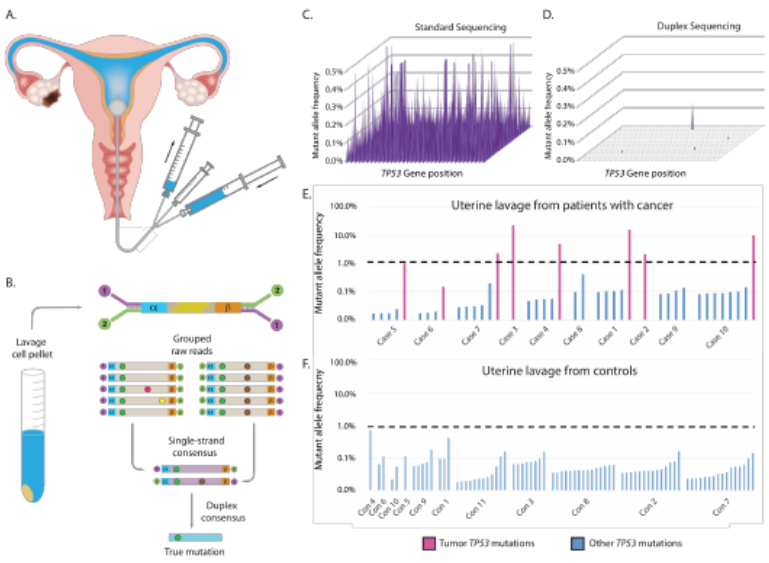


Figure 1. Detection of ovarian cancer using uterine lavage plus Duplex Sequencing. (A) Uterine lavage is carried out by passing a small catheter through the cervix followed by concurrent flushing and aspiration with 10 mL of saline as described (*24*). (B) After cell isolation by lavage centrifugation, DNA is extracted, fragmented, and ligated with specialized Duplex Sequencing adapters that include degenerate molecular tags (α and β). Following amplification, hybrid capture and sequencing, reads sharing the same barcodes are grouped into families and mutations are scored only if present in both strands of each original DNA molecule. (C) Each spot on the 2 dimensional surface represents one of the 1179 coding positions in *TP53*. The height of each peak indicates the mutant allele frequency (MAF) at each position as determined by conventional NGS, which shows false mutations at every position. (D) DS of the same sample (case 6 below) eliminates errors and reveals only true mutations. (E) *TP53* mutations identified by DS in uterine lavage from women with ovarian cancer and (F) cancer-free (controls). Fuchsia bars represent the matching tumor mutation and blue bars represent 'biological background' mutations. Mutations are sorted by ascending MAF within each patient and patients are sorted by age. Dashed lines indicate the optimal threshold to distinguish patients with and without ovarian cancer (sensitivity: 70%, specificity: 100%).

Modern conception of carcinogenesis creates opportunities to advance cancer risk assessment

Barbara L. Parsons

Current Opinion in Toxicology

Current Opinion in Toxicology 2018, 11-12:1–9
https://doi.org/10.1016/j.cotox.2018.11.003

Abstract

Tumor initiation can be viewed as abnormal tissue development. During carcinogenesis, co-localized clones of cells carrying genetic and epigenetic lesions may interact to overcome normal cellular homeostasis, setting the stage for tumor progression. This interpretation and available data support the use of some cancer driver mutations (CDMs) as reporters of carcinogenesis. Cells carrying a subset of CDMs, often referred to as hotspot CDMs, are remarkably prevalent in normal tissues, as well as in tumors, where they are detected frequently as subpopulations. Studies with model carcinogens established that geometric mean CDM mutant fractions (MFs) can serve as biomarkers of carcinogenic potential following acute or sub-chronic exposures. For some studies, the CDMs measured in tissues of treated rodents (as $\log_{10}$ MF geomean or $\log_{10}$ MF standard deviation) can be correlated with tumor responses produced by chronic exposures to the same carcinogens. Variation in cancer driver MF across rodents within a treatment group ($\log_{10}$ MF standard deviation), for a panel of CDMs, may more accurately capture the stochastic nature of

8. Summary and conclusions

The explosion in NGS technology and the knowledge of human cancer genomics derived from NGS technology should be harnessed to improve regulatory cancer risk assessment. Research efforts and investments should be directed toward advancing biologically-based rodent to human extrapolation and developing experimental approaches to derive long-term cancer risk estimates from short-term studies of exposed rodents. Hotspot CDMs are valuable biomarkers to develop in this context and the prospect of high-throughput, high-fidelity NGS methods to quantify multiple hotspot CDMs appears to be a viable and exciting path forward.

velopment, the selection of bioassay dose levels,
e scientific foundation for rodent to human
olation. Research in this area must be grounded
valid biological understanding of how CDMs
pate in carcinogenesis. One of the goals of this
therefore, is to link concepts incorporated into
s of tumorigenesis with proposed approaches
se CDMs as metrics for improving cancer risk

And, if sufficient experience were gained in relating cancer-relevant biomarkers in pre-clinical safety assessment to rodent bioassay results, it might be possible to reduce the circumstances under which bioassay data were required.

MARCH 2017 (HTTPS://WWW.NIEHS.NIH.GOV/2017/3/INDEX.HTM)

NTP Archives support global cancer research initiative

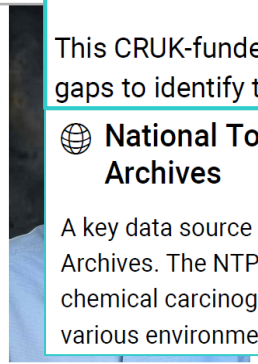
The National Toxicology Program will support a cancer and environment study that was a Cancer Research United Kingdom Grand Challenge winner.

BY VIRGINIA GUIDRY

The National Toxicology Program (NTP) Archives is contributing rodent tumor samples to a new $24.4 million study of the links between human cancers and specific environmental factors. The project is one of four winners of the Cancer Research United Kingdom (CRUK) Grand Challenge (https://www.cancerresearchuk.org/funding-for-researchers/how-we-deliver-research/grand-challenge-award?wssl=1) , announced Feb. 10.

The researchers will examine human and animal cancers for unique patterns of genetic mutations that may result from chemical exposures. These characteristic patterns are called mutational signatures, also known as mutational fingerprints. Along with expertise in rodent pathology, NTP is providing tumor and normal tissue samples from carefully documented studies of rats and mice that were exposed to more than 100 chemical carcinogens. The researchers will compare mutational fingerprints from the rodent tumors with those from human cancer tissues.

"We hope that this innovative study using the NTP Archives will help scientists better understand how substances in our environment lead to cancer," said NTP Associate Director John Bucher, Ph.D.

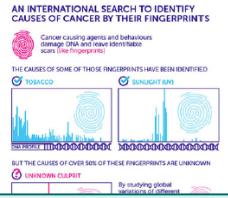


At Bucher's encouragement, the mutational fingerprints and other data generated from samples in the NTP Archives eventually will be publicly available. (Photo courtesy of Steve McCaw)

(https://www.niehs.nih.gov/2017/3/science-highlights/cancer/img811272.jpg)

Determining environmental causes of cancer

The NTP Archives are part of a project titled " Identifying Preventable Causes of Cancer (http://www.cancerresearchuk.org/funding-for-researchers/how-we-deliver-research/grand-challenge-award/funded-teams-stratton) " led by Professor Sir Mike Stratton, M.D., Ph.D., director of the Wellcome Trust Sanger Institute (WTSI). Collaborators include scientists



Mutographs of Cancer - CRUK Grand Challenge Project

This CRUK-funded Grand Challenge Project (Mutographs.org) seeks to fill in the missing gaps to identify the unknown cancer-causing factors and reveal how they lead to cancer.

National Toxicology Program (NTP) Archives

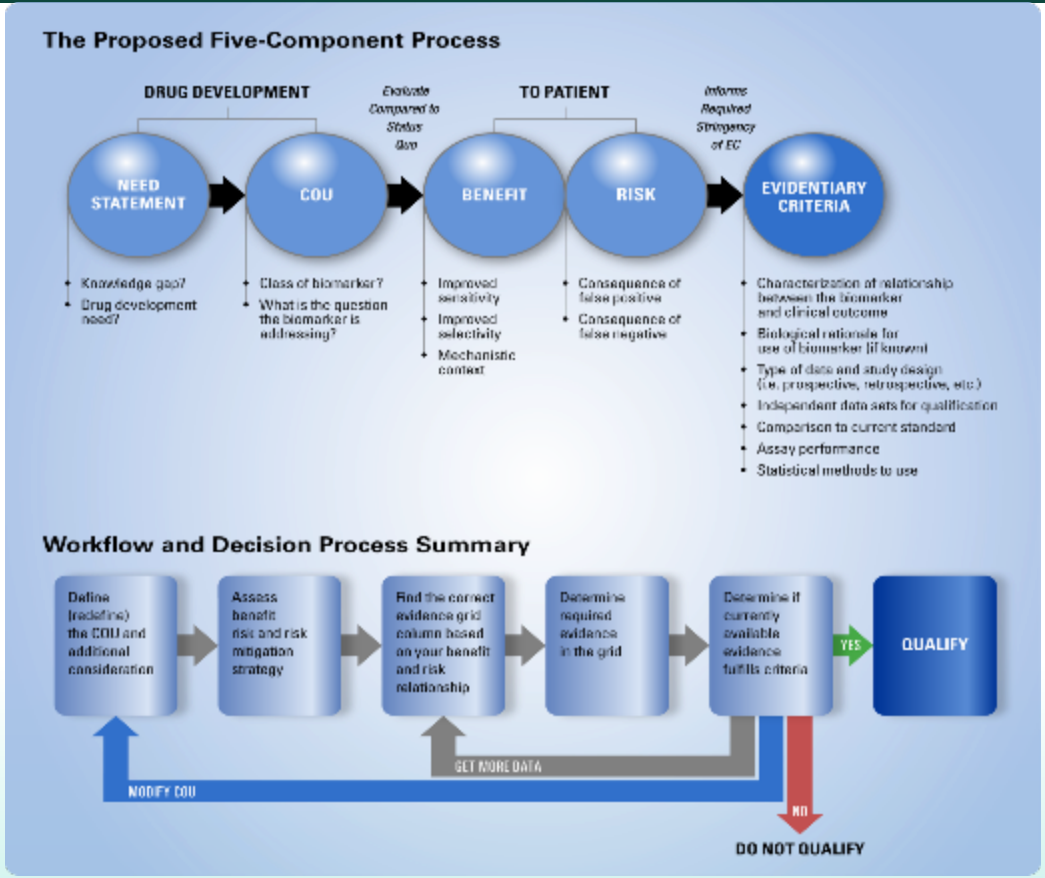
A key data source will be rat and mouse tissue samples from the National Toxicology Program (NTP) Archives. The NTP Archives is a unique repository of rodent tumor tissues exposed to more than 590 chemical carcinogens. A thorough examination of these tissues will help scientists understand how various environmental exposures may play a role in cancer.

A Dream for a Future of Pharmaceutical Carcinogenicity Evaluations of a Day Where…

- Where the molecular initiating event underlying “histologic risk factors of neoplasia” seen in a chronic rat study can be **easily** recognized, categorized, and an appropriate carco risk level aligned w DRAs…(addresses poorly explained tox findings)
- Where mode of action molecular fingerprints in tissue from the standard rat tox study can address “off target” (related target) tumorigenic risk using such novel genomic endpoints together with EC-NGS for early subclonal mutation detection… (helps address 1st in class + provide greater assurance for negatives)
- Where sponsors will apply such tools proactively early in drug development to avoid molecules and targets with high tumorigenic risk

Regulatory Authorities have Adopted an Evidentiary Criteria Framework to Advance Biomarker Qualification and Drug Development Tools

Leptak C, et al *Science Translational Medicine (2017)*



"Under the 21st Century Cures Act enacted on December 13, 2016, the new Section 507 Qualification of Drug Development Tools was added to the Federal Food, Drug, and Cosmetic Act and formally establishes an updated multistage process for biomarker qualification. This updated process includes three submission stages: the Letter of Intent, the Qualification Plan, and the Full Qualification Package."

Summary

- Ongoing ICH S1 modification negotiations are defining future opportunities for Drug Sponsors to assess pharmaceutical carcinogenicity risk without the need for conducting a two-year rat carco study on a case-by-case basis, except when essential
- Such assessments may be strengthened by leveraging genomics: transcriptomics (RNA) to identify mechanistic molecular initiating events and EC-NGS (DNA) to identify cancer driver gene mutated growth advantaged sub-clonal populations
- Consortia/world-wide collaborations have been launched to explore, evaluate, and begin momentum toward qualifying these new tools and endpoints
- Examples have been provided to explain how new genomic approaches will predict/identify/explain human relevant and irrelevant mechanisms for certain rat tumorigens to reach that future state where a two-year rat study is never needed

Acknowledgements

S1 EWG

ANVISA:	Maria Augusta Carvalho Rodrigues (Topic Leader)
BIO:	Joseph Dybowski (Topic Leader)
EFPIA:	Uli Deschl (Topic Leader)
	John Vahle (Deputy Topic Leader)
	Thomas Nolte (Expert)
EC:	Jan Willem van der Laan (EWG Regulatory Chair)
	Markku Pasanen (Topic Leader)
FDA:	Todd Bourcier (Topic Leader)
	Timothy J McGovern (Deputy Topic Leader)
Health Canada:	Alisa Vespa (Topic Leader)
	Geoffrey Ebere (Deputy Topic Leader)
JPMA:	Shigeru Hisada (Topic Leader)
	Kenjiro Tsubota (Deputy Topic Leader)
MFDS Korea:	Jong Kwon Lee (Topic Leader)
MHLW/PMDA:	Kumiko Ogawa (Topic Leader)
	Akiyoshi Nishikawa (DeputyTopic Leader)
	Mizuho Nonaka (Expert)
NMPA China:	Tao Sun (Topic Leader)
	Qingli Wang (Deputy Topic Leader)
PhRMA:	Frank D Sistare (EWG Rapporteur, Topic Leader)
	Michael Graziano (Deputy Topic Leader)
Swissmedic:	Tania Cecilia Cavaliero (Topic Leader)

Merck & Co., Inc.

- Chunhua Qin
- Alexei Podtelezhnikov
- Keith Tanis
- Alex Tamburino
- Todd Pippert
- Wendy Bailey
- Kara Pearson
- Warren Glaab
- Amy Aslamkhan

- Merck Pathology Department
- Merck Toxicology Department

- Authors of all Cited Publications and Public Databases

- Paracelsus

"All things are poisons, for there is nothing without poisonous qualities. It is only **the dose** which makes a thing a poison."