Cancer Driver Mutations in Experimental Rodents and Prediction of Human Cancer

NTP Board of Scientific Counselors Meeting
June 17, 2019

Barbara L. Parsons, PhD
Division of Genetic and Molecular Toxicology
National Center for Toxicological Research, US FDA

Long-standing unmet need for alternative approaches to the two-year rodent tumor bioassay

Current objectives:

- To develop panels of cancer driver mutational targets to serve as reporters of carcinogenic effects

Application:

- Incorporate analyses of cancer driver mutations into 28-day to 6-month repeat-dose rodent studies

Long-term goal:

- Predict rodent life-time carcinogenicity from shorter-term exposures

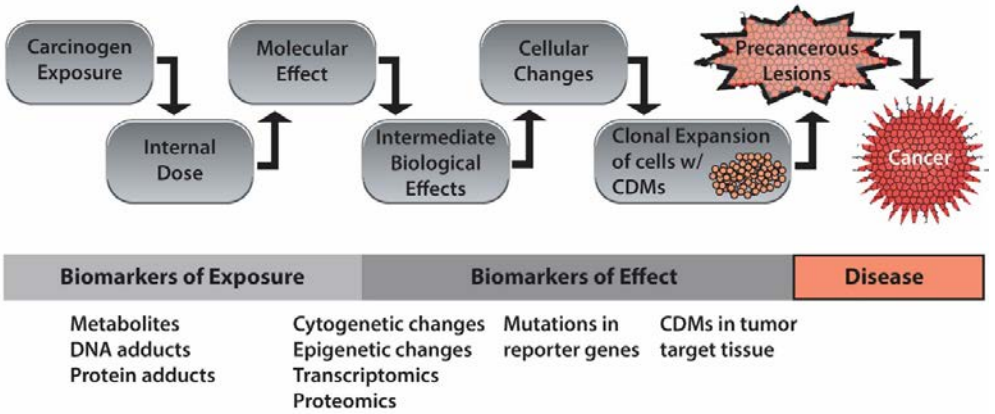
What defines cancer driver mutations (CDMs)

- Mutations causally implicated in oncogenesis
- Confer a growth advantage on the cells carrying them
- Positively-selected in the microenvironment of the tissue in which the cancer arises
- **Driver mutations tend to cause clonal expansions**

Stratton *et al.* (2009) The Cancer Genome. *Nature* 458: 719-724.

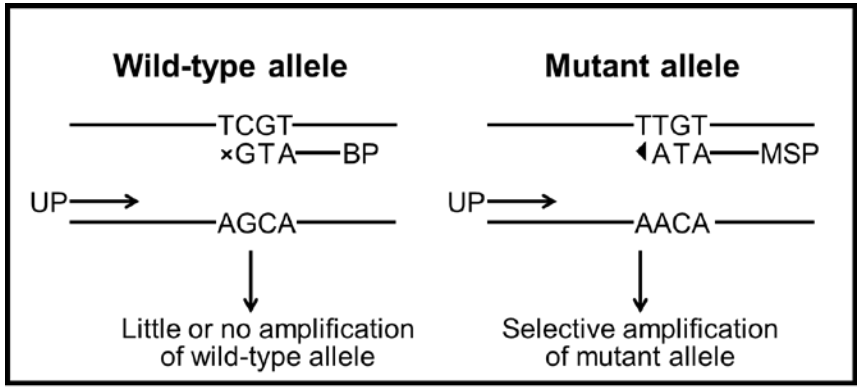
Strength of CDMs as biomarkers

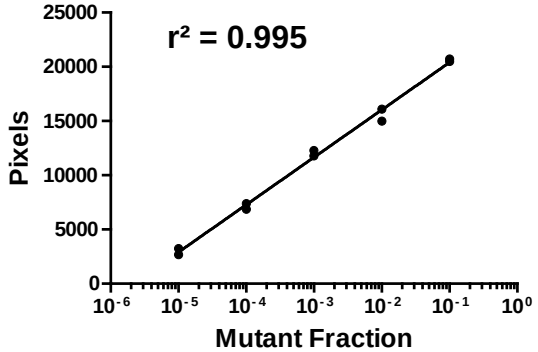
- Clonal expansion of CDMs, which is integral to tumorigenesis, will make CDMs sensitive biomarkers of effect
- Clonal expansion of CDMs is a tumor-proximate biomarker of effect
- Potential reporters for genotoxic and non-genotoxic carcinogens

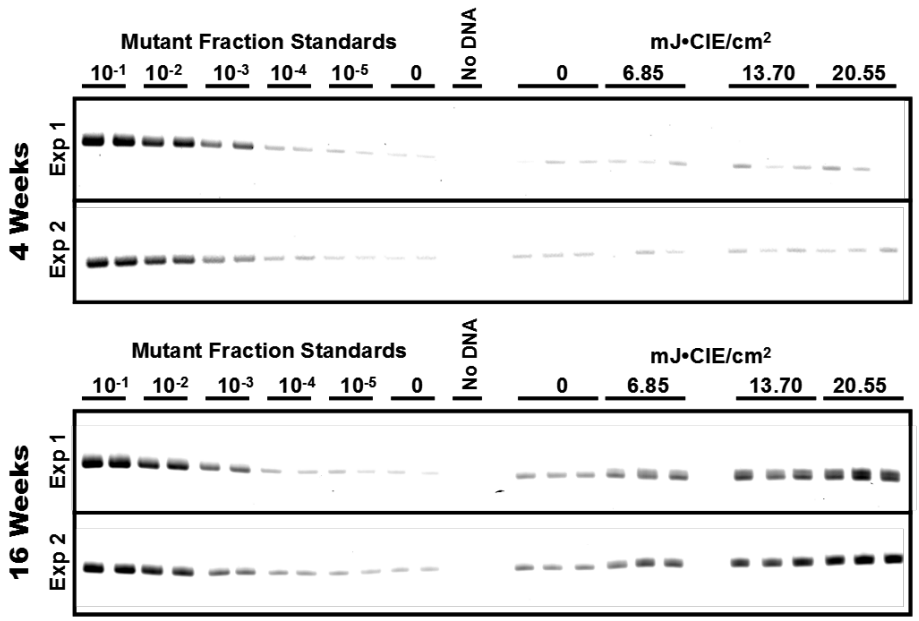


- Human Relevance
  - CDMs are being used in oncology, consequently prevalence and functional impacts of CDMs are being investigated intensively
  - NGS analyses of pre-existing clones carrying CDMs in human samples are being reported
  - DNA-based CDM measurements can be performed on any tissue of any species
  - Identify CDMs with similar tissue-specific selective advantage in human and rodents

Allele-specific competitive blocker PCR (ACB-PCR) measurement of *p53* R270C induction in mouse skin by simulated solar light (SSL)

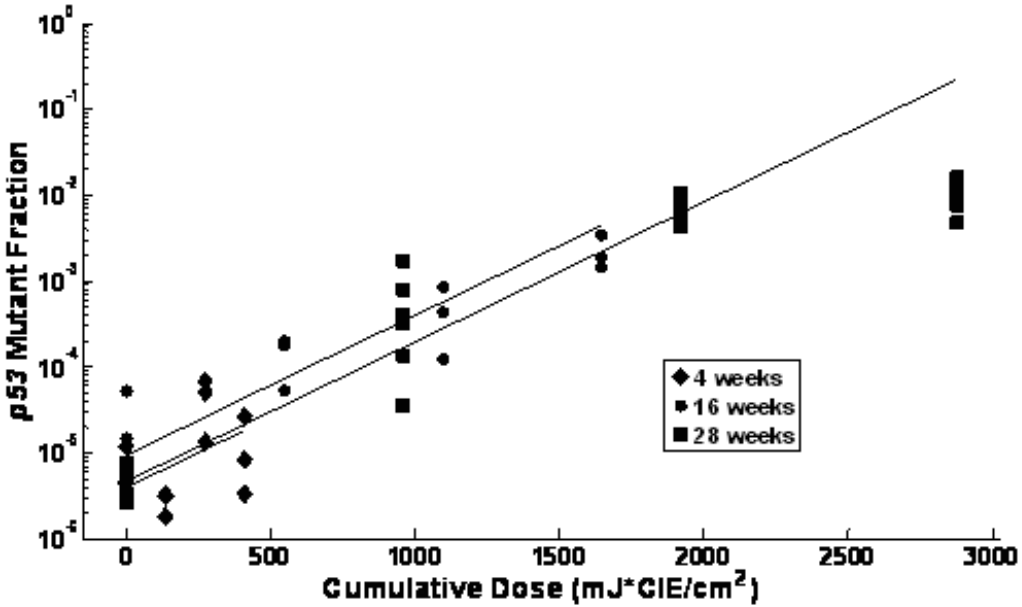






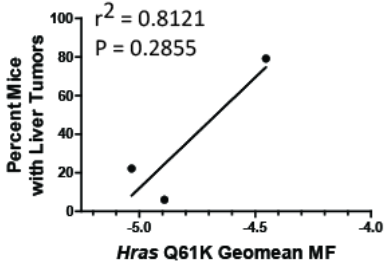
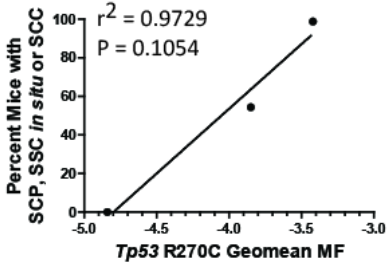
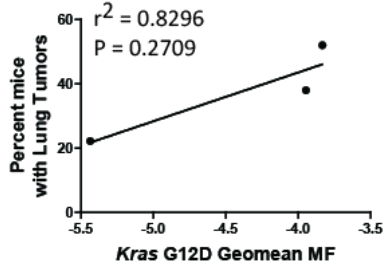
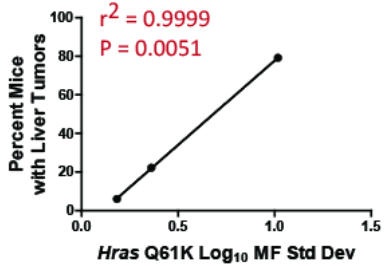
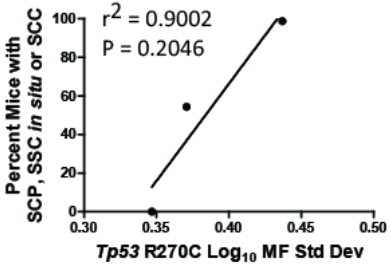
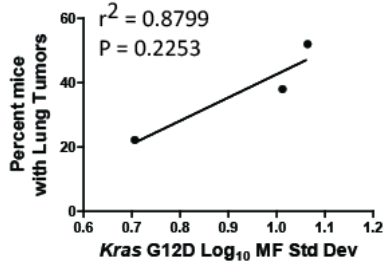
Analysis of *p53* mutations in response to dose of SSL in SKH-1 mouse skin

The increase in *p53* mutant fraction (MF) was described by the linear function: $\log_{10}MF = \alpha + 0.0016 \cdot d$, where α is the spontaneous $\log_{10}$ MF at a given timepoint and d is the dose of SSL in mJ•CIE/cm^2

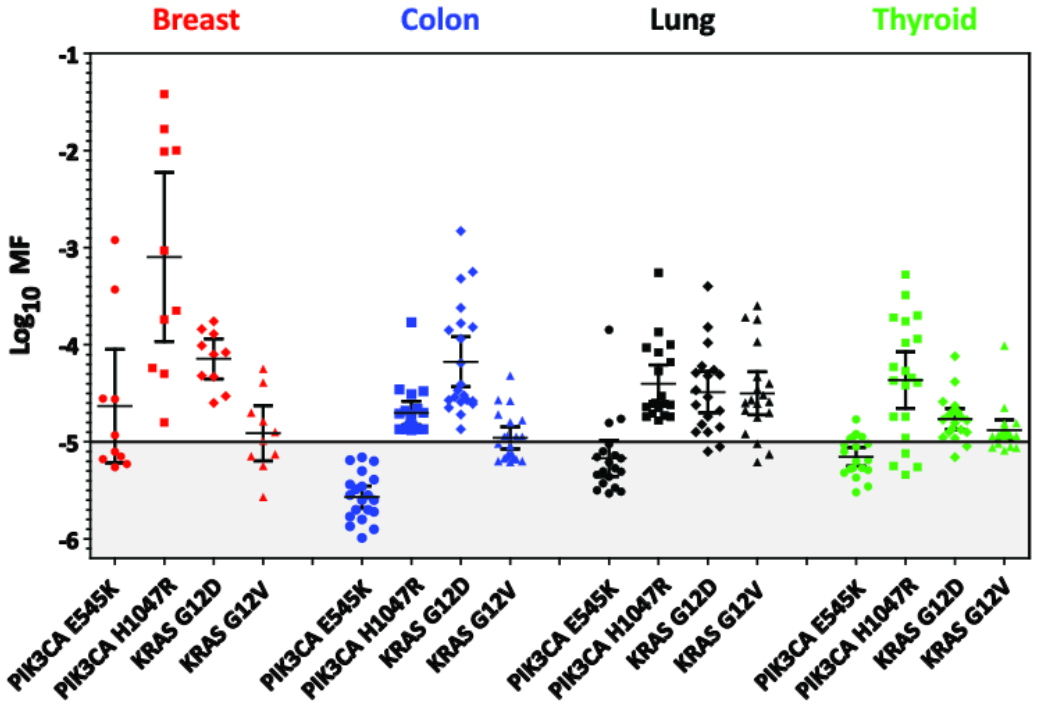


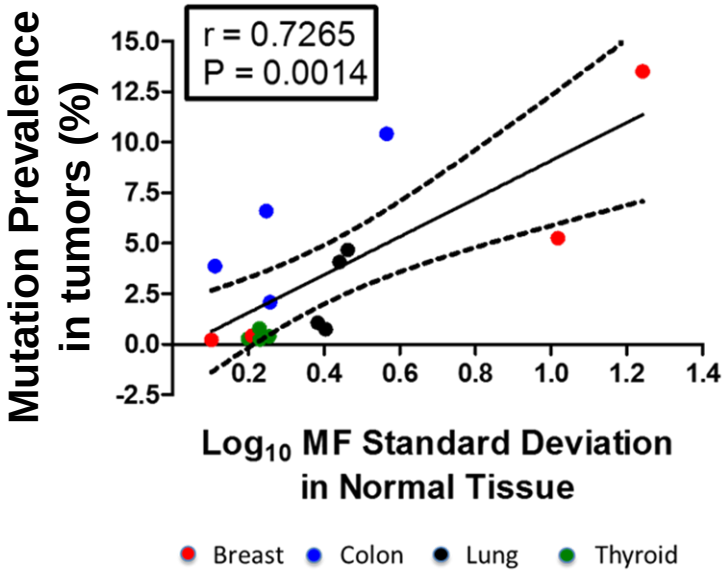
Carcinogen	Strain/species, sex, age and number of treated rodents/group	Study design, route of exposure and dosing	Induced CDM and organ analyzed	Expression period or duration for significant induction	Reference
4-Aminobiphenyl	Mouse/C57Bl/6N, male, PND 8, n=12 Mouse/B6C3F1, Male, PND 8, n=12	Acute i.p dosing, 1/3 i.p. on PND8 and 2/3 on PND15, 0 or 0.3 µmole total dose	Hras Q61K in liver	Expression time: 32 weeks	Parsons et al. (2005) Molecular Carcinogenesis 42: 193-201
N-hydroxy-2-acetylaminofluorene	Rat/Big Blue®, male, 6 weeks old, n=5 (control) n=4 (treated)	Sub-chronic, repeat dose, 4 i.p. doses of 0 or 25 mg/kg b.w. at 4-day intervals	Kras G12V Kras G12D in liver	Expression time: 10 weeks from first dose (8 from last dose)	McKinzie et al. (2006) Mutagenesis 21: 391-397
Simulated solar light	Mouse/SKH-1 hairless, female, 8 weeks old, n=3 (4 and 16 week) n=4 (28 week)	Sub-chronic, repeat dose, 5 days per week at 0, 6.85, 13.70 or 20.55 mJ·CIE/cm^2	Tp53 R270C in skin	Duration of exposure: 4, 16 or 28 weeks	Verkler et al. (2008) Molecular Carcinogenesis 47: 599-607
Benzo[a]pyrene	Mouse/A/J, male, 7-9 weeks old, n=10	Acute i.p. dosing, 0, 0.05, 0.5, or 50.0 mg/kb bw	Kras G12D Kras G12C in lung	Expression time: 4 weeks	Meng et al. (2010) Environ. Mol. Mutagen. 51: 145-155
Azoxymethane	Rats/F344, male, 7 weeks old, n=6	Acute s.c. dosing 0 or 30 mg/kg bw, ½ at week 7, ½ at week 8	Kras G12V Kras G12D in colon	Expression times: 1, 8, 24 or 32 weeks	McKinzie et al. (2011) Environ. Mol. Mutagen. 52: 409-418
Aristolochic acid	Rat/Big Blue®, male, 6 weeks old, n=6	Sub-chronic, repeat dose, 5 days per week at 0, 0.1, 1.0, and 10.0 mg/kg bw by gavage	Kras G12D in kidney Hras Q61L in liver and kidney	Duration of exposure: 12 weeks	Wang et al. (2011) Mutagenesis 26: 619-628
Aristolochic acid	Mouse/Hupki, female, 2-4 months old, n=3 (3 or 12 days) n=5 (21 days)	Acute/sub-chronic, repeat dose, daily at 0 or 5 mg/kg bw	Hras Q61L in kidney and forestomach	Duration of exposure: 3 weeks	Wang et al. (2012) Environ. Mol. Mutagen. 53: 495-504
Ethylene oxide	Mouse/Big Blue®, male, 8 weeks old, n=10	Sub-chronic, repeat dose, 6 hours per day, 5 days per week at 0, 10, 50, 100 and 200 ppm by inhalation	Kras G12V Kras G12D in lung	Duration of exposure: 4 weeks	Parsons et al. (2013) Toxicological Sciences 136: 26-38

Relationships between cancer driver MFs and tumor responses

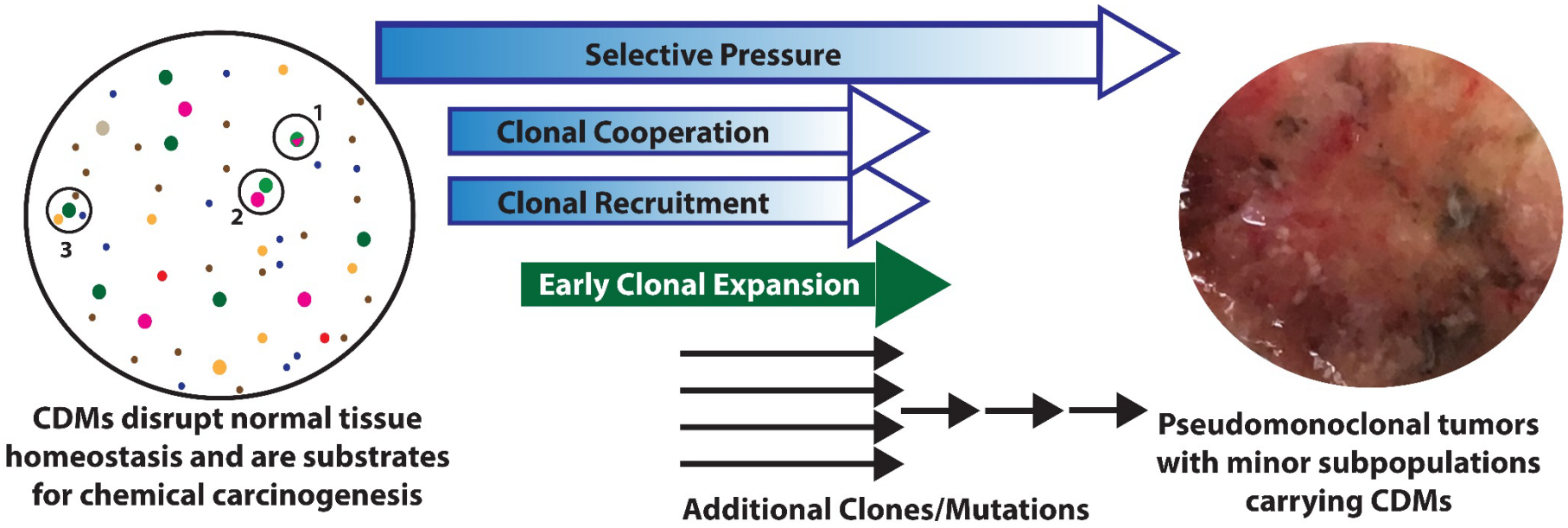
	Study 1 4-aminobiphenyl 8 month expression time vs tumors @ 1 yr newborn mouse assay design: 0.3 umol 4-ABP- or DMSO-treated B6C3F$_1$ and DMSO-treated C57Bl/6 mice	Study 2 Simulated solar light 4 month repeat dose vs tumors @ 1 yr 0, 6.85 and 13.70 mJ·CIE/cm2 SKH-1 mice	Study 3 Inhaled ethylene oxide 1 month repeat dose vs lung tumors @ 2 yrs 0, 50 or 100 ppm in B6C3F$_1$ BigBlue mice
Log$_{10}$ Geomean MF			
Log$_{10}$ MF Standard Deviation			

CDMs are prevalent in normal tissues and interindividual variation identifies tissue-specific selective advantage

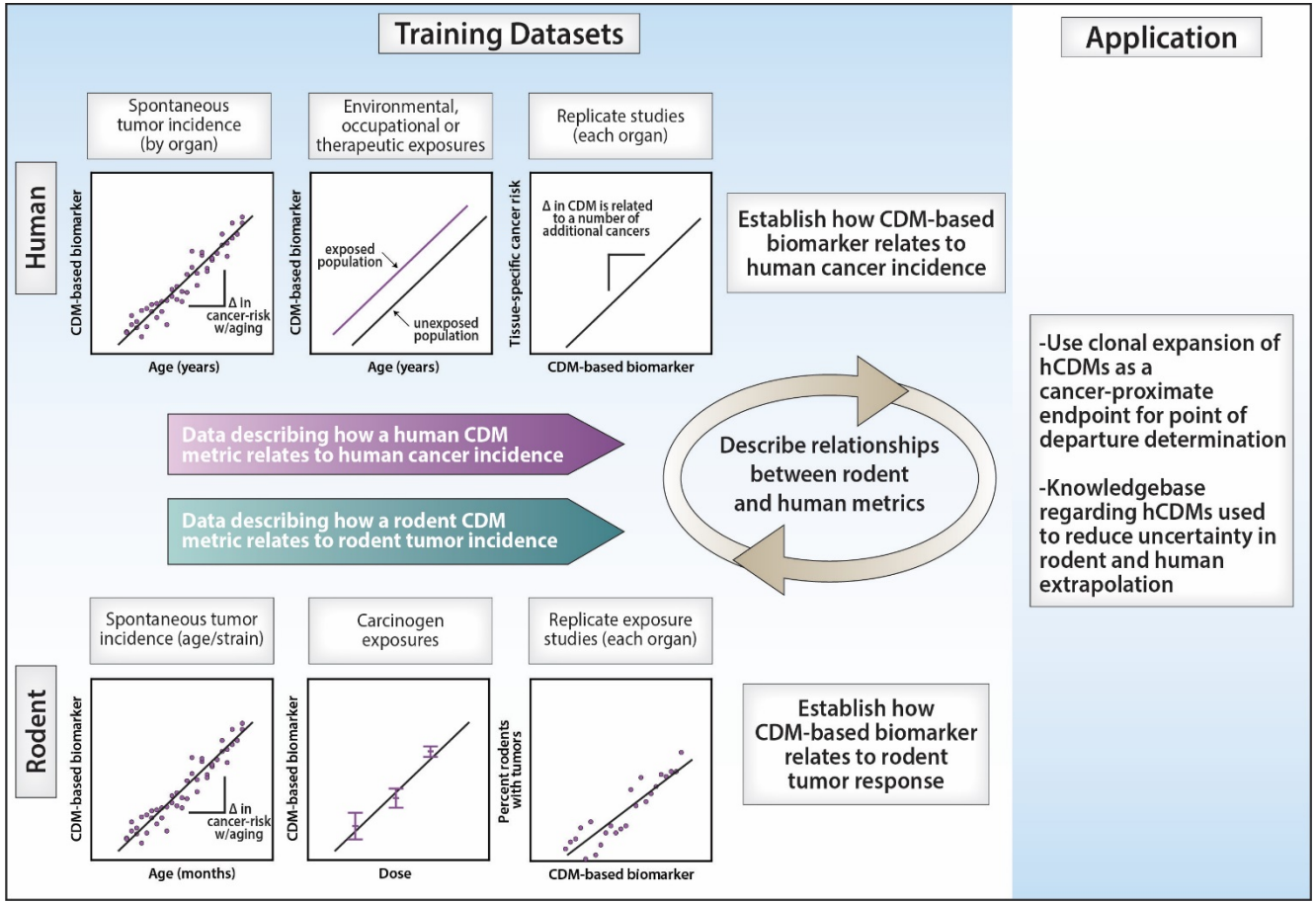




CDMs as biomarkers in a field cancerization view of carcinogenesis



The path forward



Need datasets defining relationships between measurements from panels of hotspot CDMs and tumor responses for rodent and human

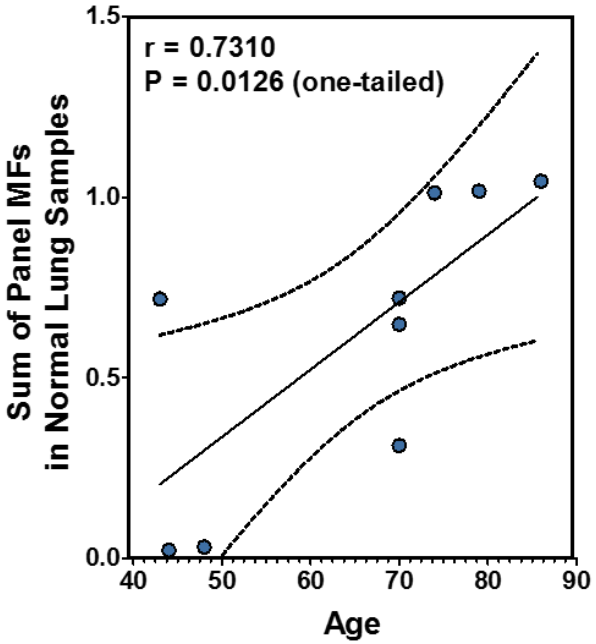
Developed a human amplicon panel and analysis by error-corrected NGS (EC-NGS)

Gene	Mutation Prevalence in Breast Ductal Carcinoma*	Mutation Prevalence in Lung Adenocarcinoma*	Exon	Amino Acid Substitution	Mutation
TP53	40%	38%	5	R175H	G>A
			7	R248Q	G>A
			7	R248W	C>T
			8	R273H	G>A
			8	R273C	C>T
EGFR	1.3%	31%	19	T790M	C>T
			21	L858R	T>G
KRAS	1.1%	19%	2	G12C	G>T
			2	G12A	G>C
			2	G12D	G>A
			2	G12R	G>C
			2	G12S	G>A
			2	G12V	G>T
			2	G13D	G>A
			2	R80*	C>T
STK11	<1%	10%	8	F354L	C>G
SETBP1	<1%	7%	4	D868N	G>A
			4	G870S	G>A
			4	I871T	T>C
NFE2L2	<1%	1.3%	2	D29H	G>C
			2	D27G	A>G
			2	R34Q	G>A
			2	E79K	G>A
			2	E79Q	G>C
			2	T80P	A>C
			2	G81D	G>A
			2	E82Q	G>C
			2	E82D	A>T
PIK3CA	24%	3.9%	9	E542K	G>A
			9	E545K	G>A
			20	H1047R	A>G
BRAF	<1%	2.6%	15	V600E	T>A
APC	2.1%	3.8%	15	R1450*	C>T

- The panel contains 13 amplicons from 9 cancer driver genes - 973 bp with >30 mutational hotspots
- Amplicons conserved coding sequences (human, rat, and mouse)
- Analyzed 9 normal lung, 9 normal breast, 10 ductal carcinomas and 9 lung adenocarcinomas
- Unique molecular identifier sequences incorporated during a first-round PCR
- A single-strand consensus sequence approach used for error correction

Performance of a human EC-NGS method

- Sensitivity of 10^{-4}
- Good correlation (Spearman r = 0.9371, P <0.0001, n = 28) and no bias (Bland-Altman Log_{10} bias = 0.00288) between ACB-PCR and EC-NGS MF measurements
- 50-100 mutations detected/sample, including expected hotspot CDMs and other COSMIC missense mutations
- Expected tissue-specific mutant enrichment for both tumor and normal
- Significant differences in mutational spectra and observed the expected predominant mutational specificities for breast and lung carcinomas
- Significant correlation between sum of MFs for each individual and tissue donor age



Conclusions

- Panels of hotspot CDMs appear to be promising biomarkers of carcinogenic effect and may be translatable between rodent and human
- Measurements of CDMs can simultaneously serve as reporters of carcinogenic effect and discern differences in mutation spectra
- Moving forward, need to define relationships between panels of CDM measurements and tumor responses for rodent and human
- Envision using panels of CDMs in a tissue-specific manner, during pre-clinical safety assessment of drugs or repeat-dose toxicity testing, focusing on tissues where pharmacological impacts are expected or toxicity is observed.
- Currently, developing rat and mouse amplicon panels analogous to human
  - Validating rodent panels using rodents with known strain differences in spontaneous mammary and lung tumor susceptibility

Acknowledgements

NCTR Collaborators

ACB-PCR

Division of Genetic and Molecular Toxicology
- Meagan B. Myers
- Karen L. McKim
- Yiying Wang
- Fanxue Meng
- Tracie Verkler
- Page McKinzie
- Nan Mei
- Tao Chen
- Manju Manjanatha
- Robert Heflich
- Martha Moore

Division of Biochemical Toxicology
- Fred Beland
- Paul Howard

Division of Bioinformatics and Biostatistics
- Robert Delongchamp

EC-NGS

Division of Genetic and Molecular Toxicology
- Kelly Harris
- Vijay Walia, Commissioner's Fellow
- Karen L. McKim
- Meagan Myers

Division of Bioinformatics and Biostatistics
- Binsheng Gong
- Joshua Xu

External Collaborators

Jeffrey Ross, EPA
Volker Arlt, King's College London
David Phillips, King's College London
Bhaskar Gollapudi, Dow Chemical Company
Nigel Moore, Dow Europe GmbH
Lynne Haber, TERA
Timothy Robison, FDA, CDER