"The Meso Gene"
Evaluating Predispositions to Certain Types of Cancer

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SCIENTIFIC CONSULTANTS to INDUSTRY and COUNSEL

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Why Genetic Science in Asbestos Cases?

Separate Exposure-induced Disease
from Idiopathic Disease

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Presentation Overview

- Some Genomics Basics
- BAP1 and Mesothelioma
- Other Mutations Linked to Mesothelioma
- Genetics in Talc and Ovarian Cancer

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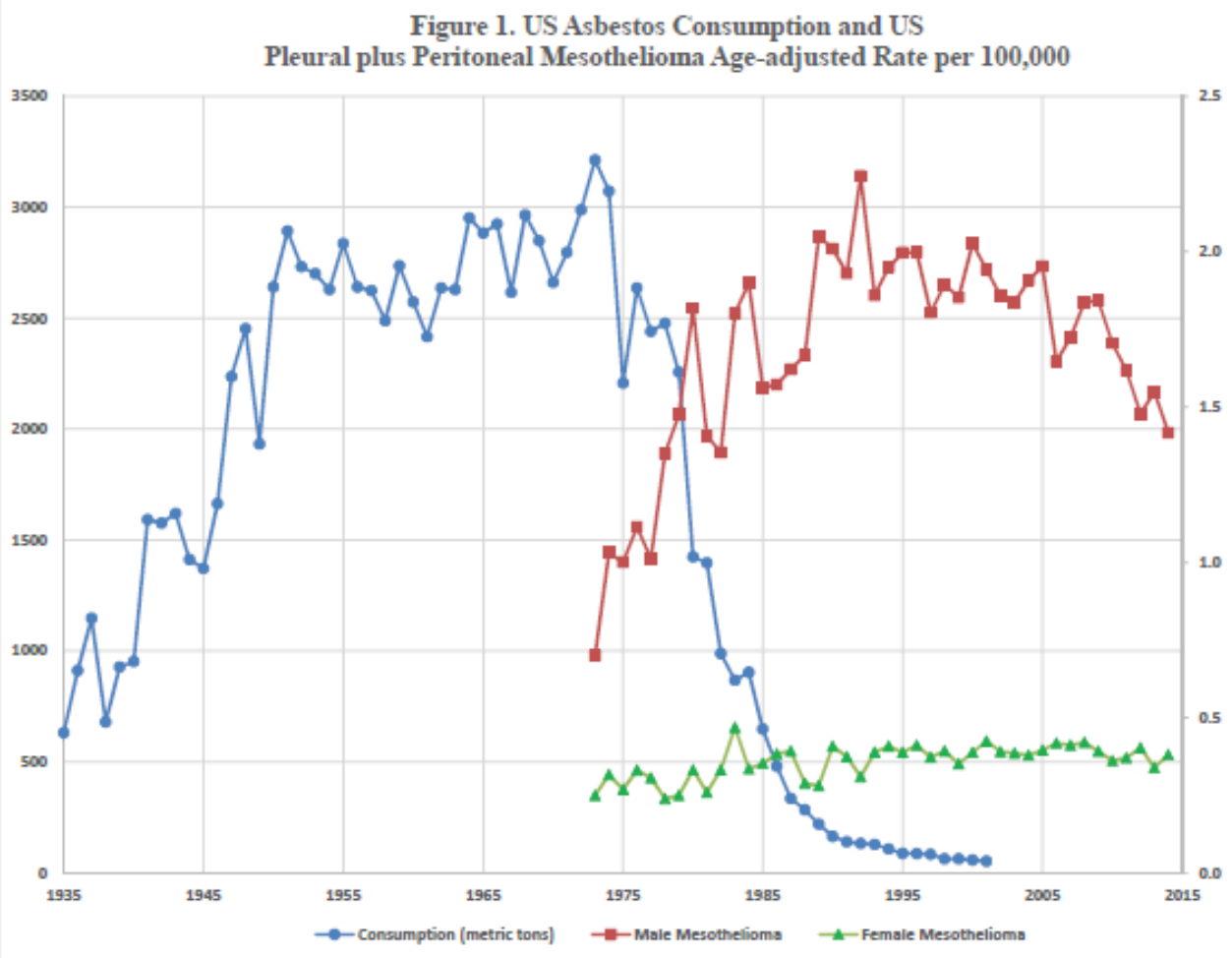
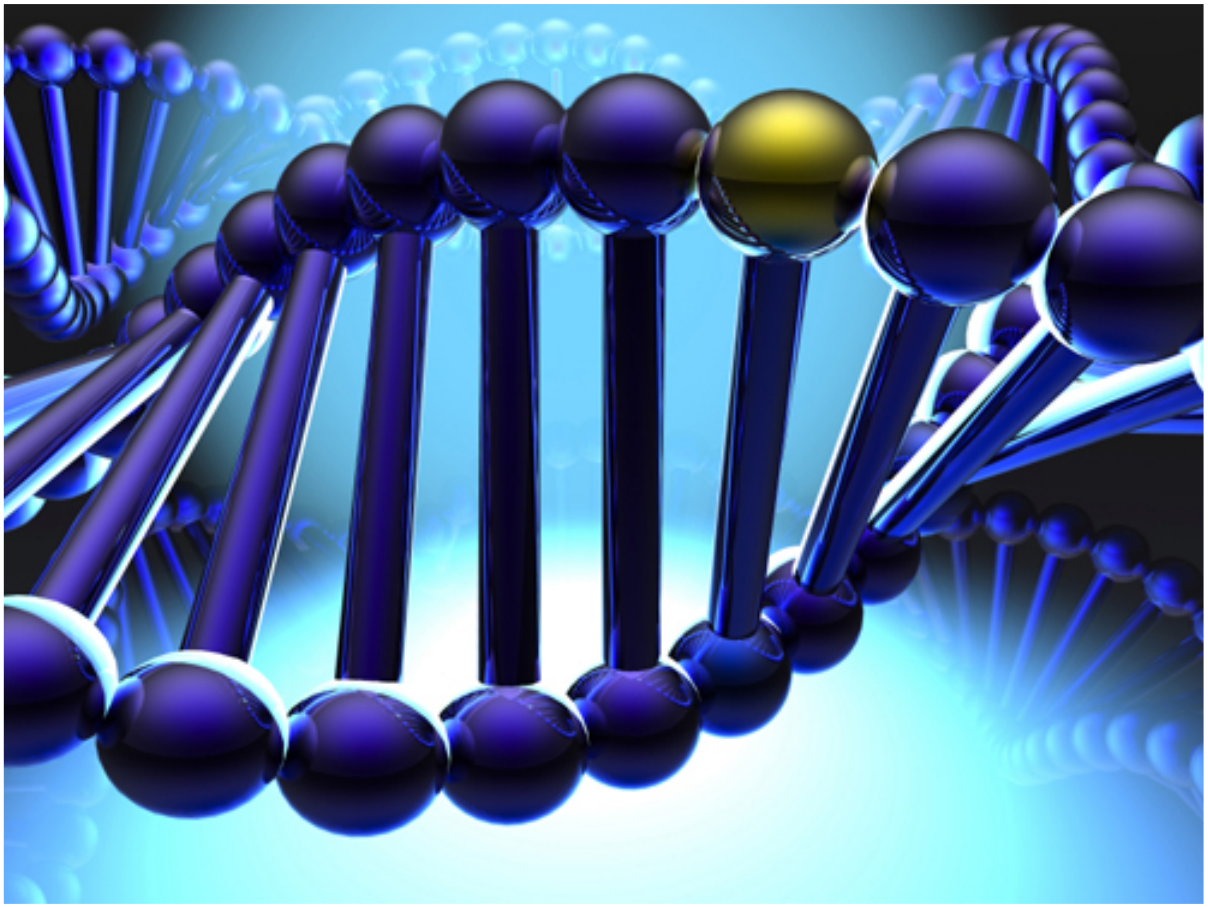
Asbestos Exposure is Plummeting… Yet Meso Cases are Steady

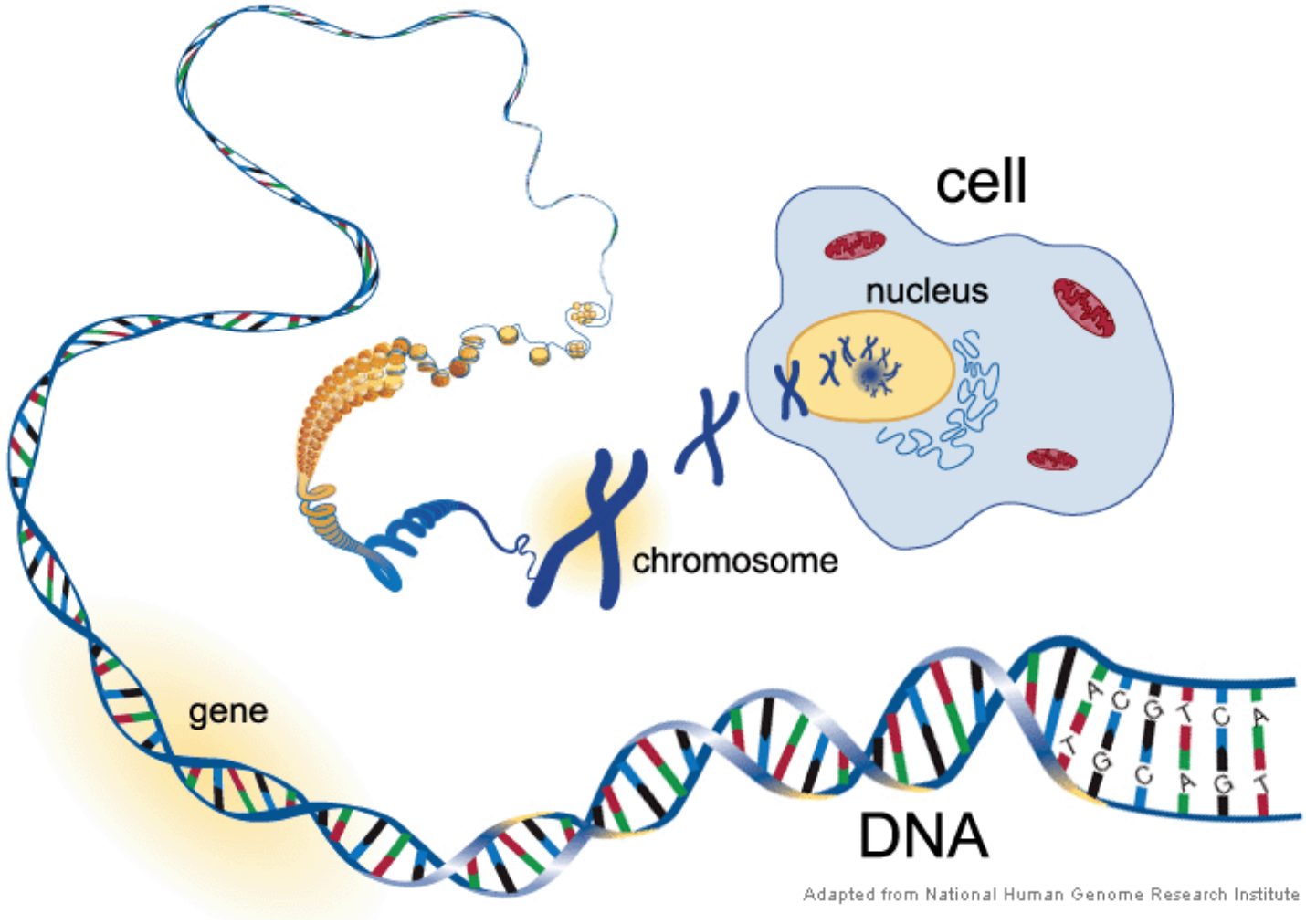


Some Genomics Basics

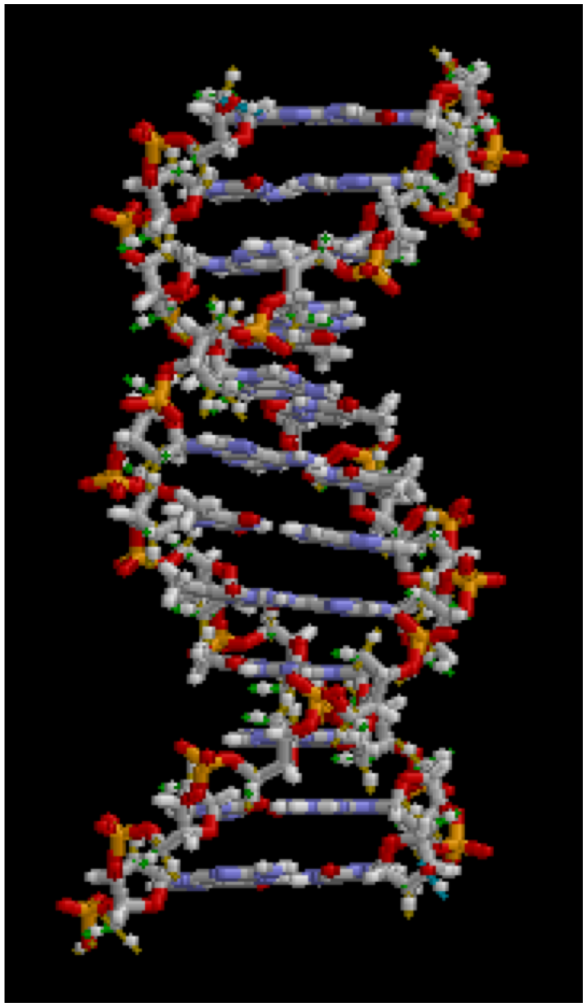
Cancer is a Disease of the Genome

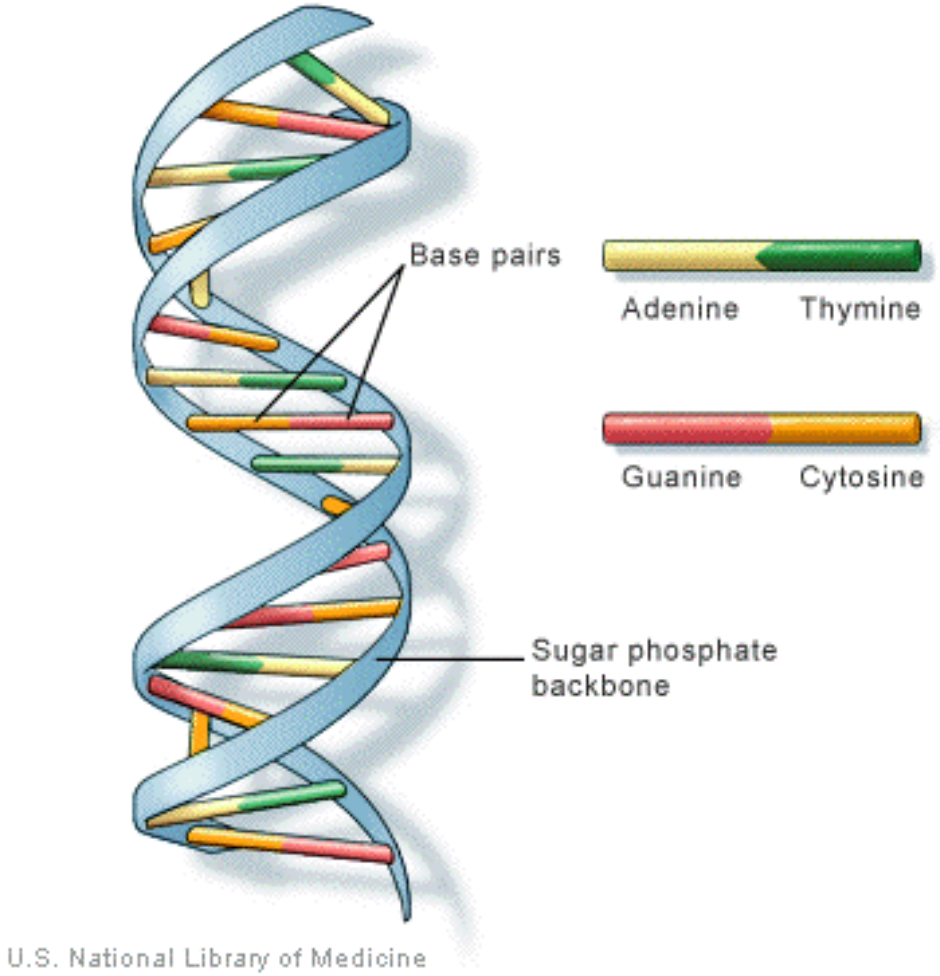


The Human Genome



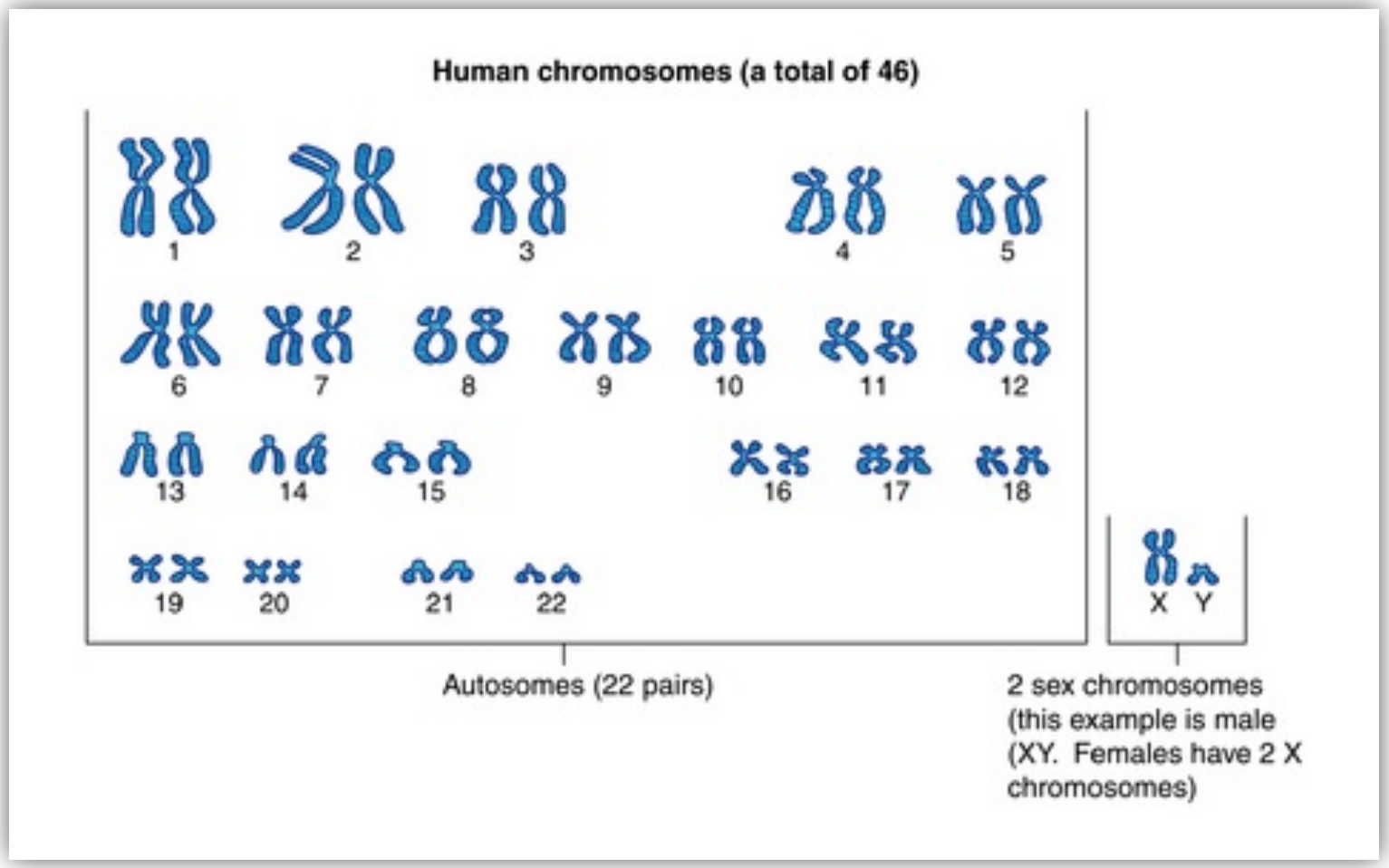
DNA





U.S. National Library of Medicine

23 Pair of Chromosomes

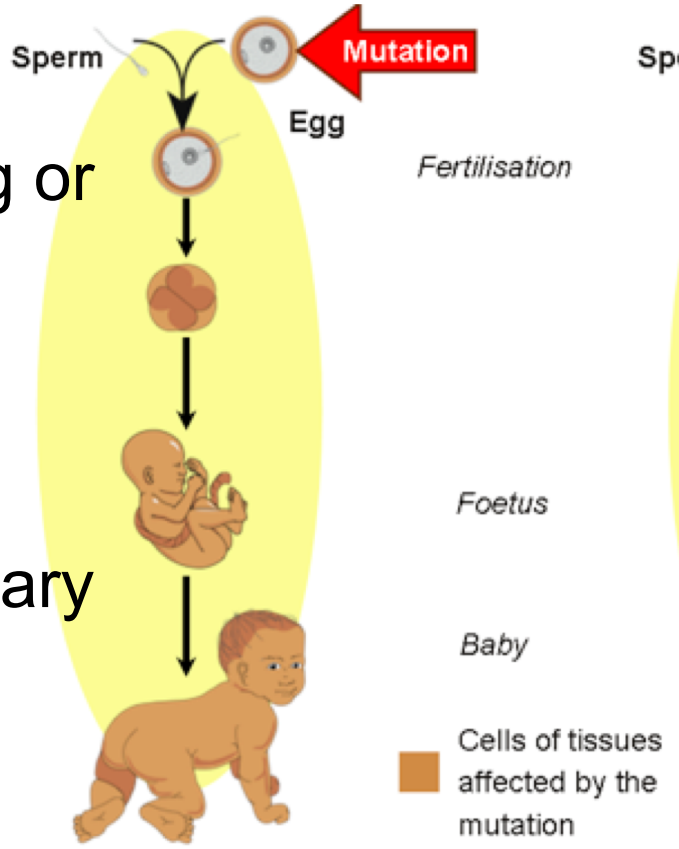


Germline Mutations

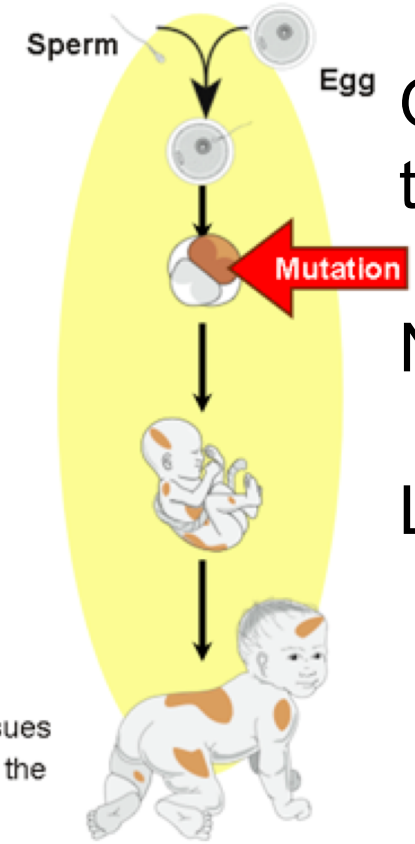
Present in egg or sperm

Heritable

Cause hereditary cancer syndromes



Somatic Mutations



Occur in cancer tissues

Non-heritable

Later onset

Predisposition vs. Susceptibility

- **Genetic Predisposition**
  - A genotype that increases likelihood of developing a disease
  - No toxin required
  - Not every carrier of a predisposing genetic variant(s) will get the disease

- **Genetic Susceptibility**
  - A genotype that increases the likelihood of a toxin causing a disease
  - Individuals can be susceptible or resistant (have protective factors)

Predisposition vs. Susceptibility

Pro-Plaintiff

- Toxin-induced disease
- Toxin-induced mutation
- Eggshell Plaintiff

Intermediate

- Inherited mutation **may** increase susceptibility
- Inherited mutation **may** predispose toward injury

Pro-Defense

- Inherited mutation caused the injury
- High penetrance
- Alternative cause argument

Pure Susceptibility ⟷ **Pure Predisposition**

Genomics Basics – Key Concepts

- Cancer is a disease of the genome
- Your genetic sequence is encoded in your DNA
- There are two broad types of mutations
  - Germline
  - Somatic
- Predisposition vs. Susceptibility
  - Genetic predisposition is when a genotype increases risk of disease **in absence of exposure**
  - Genetic susceptibility is when a genotype increases risk of **exposure-induced disease**

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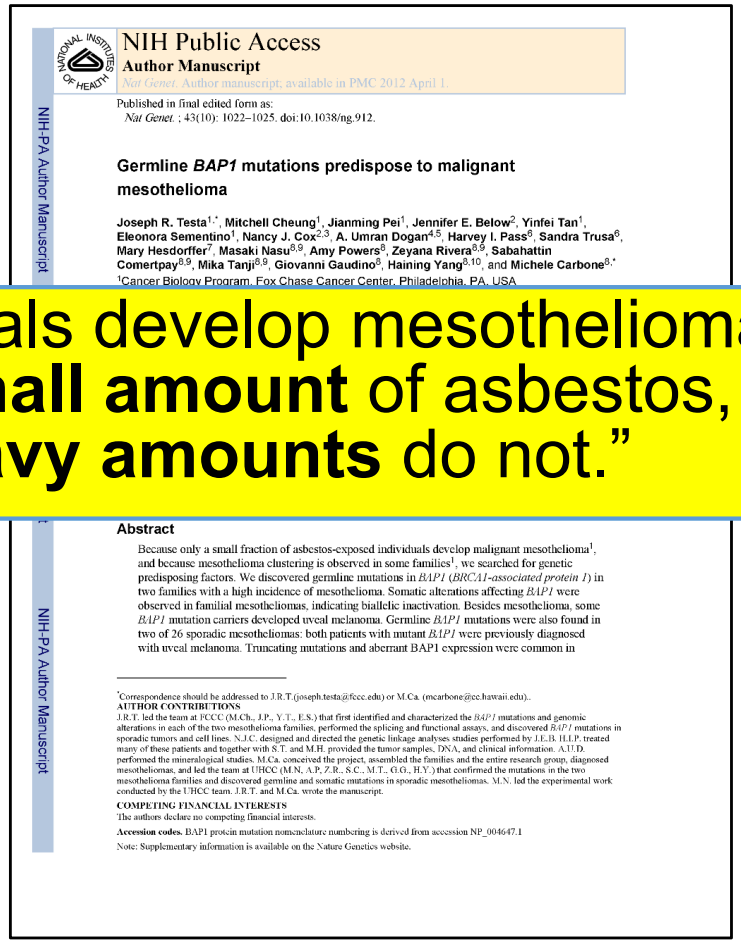
BAP1 and Mesothelioma

Some Get Mesothelioma -- Others Do Not



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Germline *BAP1* mutations predispose to malignant mesothelioma

Joseph R. Testa[1,*], **Mitchell Cheung**[1], **Jianming Pei**[1], **Jennifer E. Below**[2], **Yinfei Tan**[1], **Eleonora Sementino**[1], **Nancy J. Cox**[2,3], **A. Umran Dogan**[4,5], **Harvey I. Pass**[6], **Sandra Trusa**[6], **Mary Hesdorffer**[7], **Masaki Nasu**[8,9], **Amy Powers**[8], **Zeyana Rivera**[8,9], **Sabahattin Comertpay**[8,9], **Mika Tanji**[8,9], **Giovanni Gaudino**[6], **Haining Yang**[8,10], and **Michele Carbone**[6,*]
[1]Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA, USA

NIH-PA Author Manuscript

"Some individuals develop mesothelioma following exposure to **small amount** of asbestos, while others exposed to **heavy amounts** do not."

Abstract

Because only a small fraction of asbestos-exposed individuals develop malignant mesothelioma[1], and because mesothelioma clustering is observed in some families[1], we searched for genetic predisposing factors. We discovered germline mutations in *BAP1* (*BRCA1-associated protein 1*) in two families with a high incidence of mesothelioma. Somatic alterations affecting *BAP1* were observed in familial mesotheliomas, indicating biallelic inactivation. Besides mesothelioma, some *BAP1* mutation carriers developed uveal melanoma. Germline *BAP1* mutations were also found in two of 26 sporadic mesotheliomas: both patients with mutant *BAP1* were previously diagnosed with uveal melanoma. Truncating mutations and aberrant BAP1 expression were common in

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AUTHOR CONTRIBUTIONS

J.R.T. led the team at FCCC (M.Ch., J.P., Y.T., E.S.) that first identified and characterized the *BAP1* mutations and genomic alterations in each of the two mesothelioma families, performed the splicing and functional assays, and discovered *BAP1* mutations in sporadic tumors and cell lines. N.J.C. designed and directed the genetic linkage analyses studies performed by J.E.B. H.I.P. treated many of these patients and together with S.T. and M.H. provided the tumor samples, DNA, and clinical information. A.U.D. performed the mineralogical studies. M.Ca. conceived the project, assembled the families and the entire research group, diagnosed mesotheliomas, and led the team at UHCC (M.N, A.P, Z.R., S.C., M.T., G.G., H.Y.) that confirmed the mutations in the two mesothelioma families and discovered germline and somatic mutations in sporadic mesotheliomas. M.N. led the experimental work conducted by the UHCC team. J.R.T. and M.Ca. wrote the manuscript.

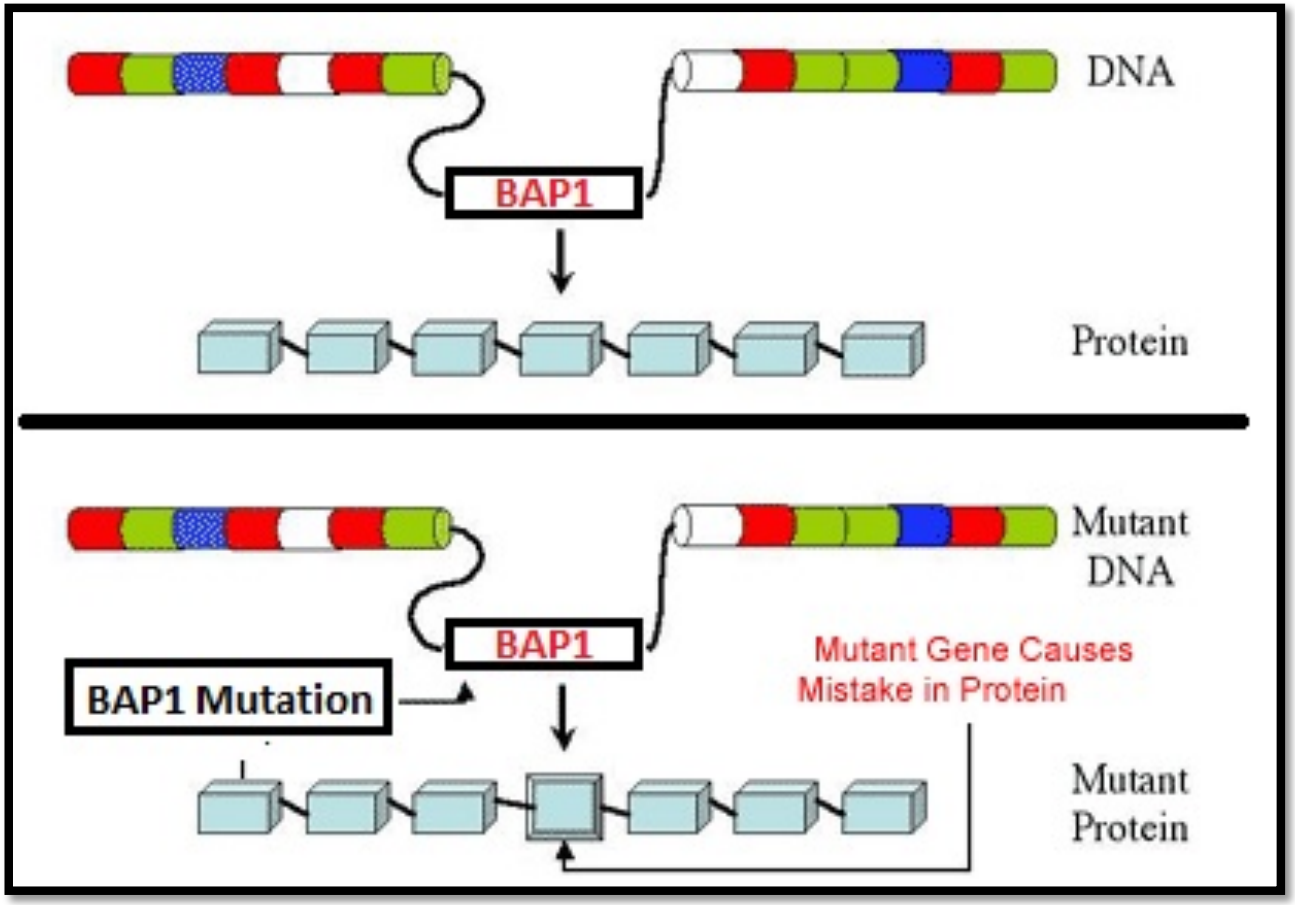
COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Accession codes. BAP1 protein mutation nomenclature numbering is derived from accession NP_004647.1

Note: Supplementary information is available on the Nature Genetics website.

BAP1 Mutations Produce Ineffective BAP1 Protein



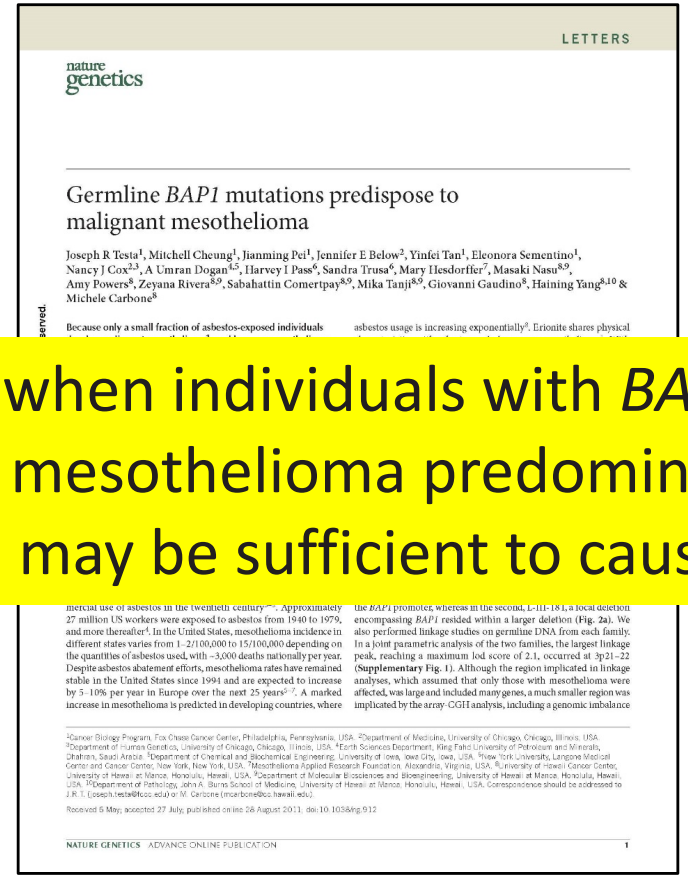
Normal BAP1 Protein

- Regulates cell replication
- Controls DNA repair
- Controls gene activity
- Tumor suppressor

Mutated BAP1 Protein

- Regu[illegible]s ce[illegible]eplication
- Controls [illegible]A repair
- Contro[illegible]g[illegible]e activity
- Tum[illegible] suppr[illegible]ssor

Predisposition or Susceptibility?



LETTERS

nature genetics

Germline *BAP1* mutations predispose to malignant mesothelioma

Joseph R Testa[1], Mitchell Cheung[1], Jianming Pei[1], Jennifer E Below[2], Yinfei Tan[1], Eleonora Sementino[1], Nancy J Cox[2,3], A Umran Dogan[4,5], Harvey I Pass[6], Sandra Trusa[6], Mary Hesdorffer[7], Masaki Nasu[8,9], Amy Powers[8], Zeyana Rivera[8,9], Sabahattin Comertpay[8,9], Mika Tanji[8,9], Giovanni Gaudino[8], Haining Yang[8,10] & Michele Carbone[8]

Because only a small fraction of asbestos-exposed individuals

asbestos usage is increasing exponentially[9]. Erionite shares physical

mercial use of asbestos in the twentieth century[2–5]. Approximately 27 million US workers were exposed to asbestos from 1940 to 1979, and more thereafter[4]. In the United States, mesothelioma incidence in different states varies from 1–2/100,000 to 15/100,000 depending on the quantities of asbestos used, with ~3,000 deaths nationally per year. Despite asbestos abatement efforts, mesothelioma rates have remained stable in the United States since 1994 and are expected to increase by 5–10% per year in Europe over the next 25 years[2–7]. A marked increase in mesothelioma is predicted in developing countries, where

the *BAP1* promoter, whereas in the second, L-III-18.1, a local deletion encompassing *BAP1* resided within a larger deletion (**Fig. 2a**). We also performed linkage studies on germline DNA from each family. In a joint parametric analysis of the two families, the largest linkage peak, reaching a maximum lod score of 2.1, occurred at 3p21–22 (**Supplementary Fig. 1**). Although the region implicated in linkage analyses, which assumed that only those with mesothelioma were affected, was large and included many genes, a much smaller region was implicated by the array-CGH analysis, including a genomic imbalance

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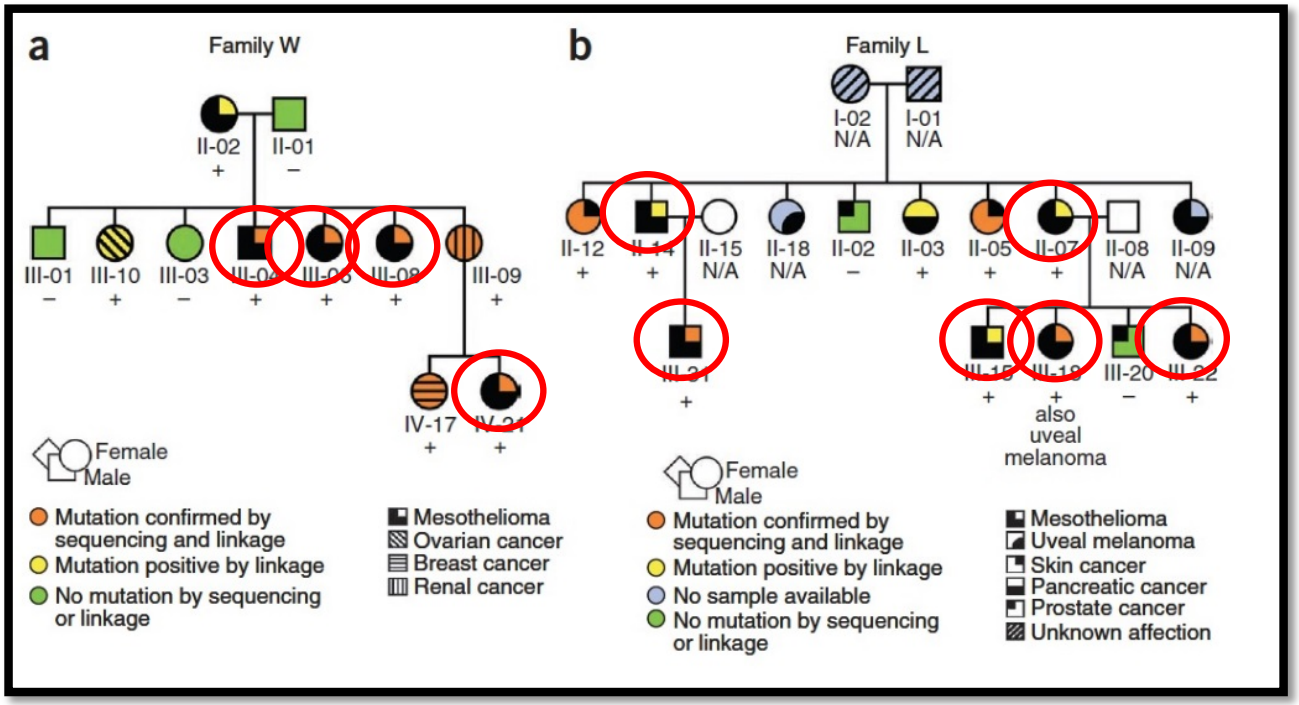
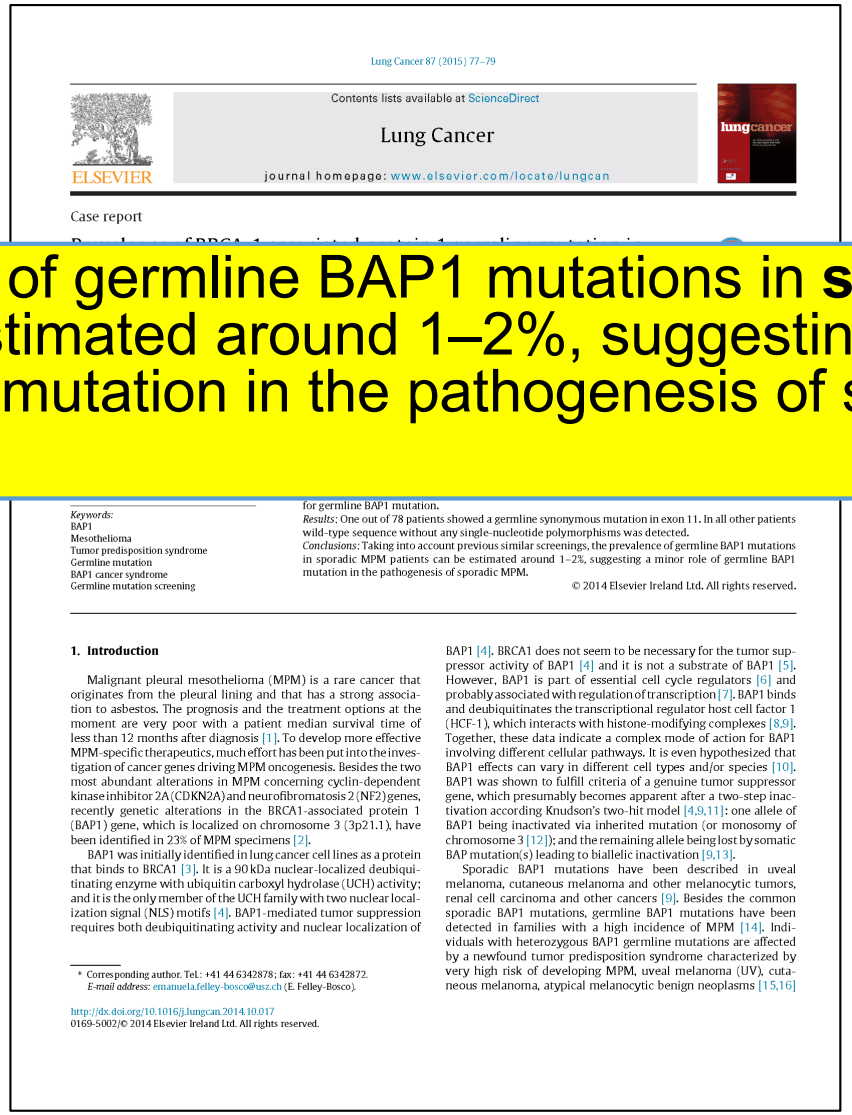
We hypothesize that when individuals with *BAP1* mutations are exposed to asbestos, mesothelioma predominates. Alternatively, *BAP1* mutation alone may be sufficient to cause mesothelioma.

Evidence for Genetic Predisposition
BAP1 Mutations Predispose Families to MM



Testa JR, et al. Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet. 2011 Aug 28;43(10):1022-5.

BAP1 Mutations Drive Familial MM More than Sporadic MM



Lung Cancer 87 (2015) 77–79

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Case report

"…the prevalence of germline BAP1 mutations in **sporadic MPM** patients can be estimated around 1–2%, suggesting a minor role of germline BAP1 mutation in the pathogenesis of sporadic MPM."

Keywords:
BAP1
Mesothelioma
Tumor predisposition syndrome
Germline mutation
BAP1 cancer syndrome
Germline mutation screening

for germline BAP1 mutation.
Results: One out of 78 patients showed a germline synonymous mutation in exon 11. In all other patients wild-type sequence without any single-nucleotide polymorphisms was detected.
Conclusions: Taking into account previous similar screenings, the prevalence of germline BAP1 mutations in sporadic MPM patients can be estimated around 1–2%, suggesting a minor role of germline BAP1 mutation in the pathogenesis of sporadic MPM.

1. Introduction

Malignant pleural mesothelioma (MPM) is a rare cancer that originates from the pleural lining and that has a strong association to asbestos. The prognosis and the treatment options at the moment are very poor with a patient median survival time of less than 12 months after diagnosis [1]. To develop more effective MPM-specific therapeutics, much effort has been put into the investigation of cancer genes driving MPM oncogenesis. Besides the two most abundant alterations in MPM concerning cyclin-dependent kinase inhibitor 2A (CDKN2A) and neurofibromatosis 2 (NF2) genes, recently genetic alterations in the BRCA1-associated protein 1 (BAP1) gene, which is localized on chromosome 3 (3p21.1), have been identified in 23% of MPM specimens [2].

BAP1 was initially identified in lung cancer cell lines as a protein that binds to BRCA1 [3]. It is a 90 kDa nuclear-localized deubiquitinating enzyme with ubiquitin carboxyl hydrolase (UCH) activity; and it is the only member of the UCH family with two nuclear localization signal (NLS) motifs [4]. BAP1-mediated tumor suppression requires both deubiquitinating activity and nuclear localization of BAP1 [4]. BRCA1 does not seem to be necessary for the tumor suppressor activity of BAP1 [4] and it is not a substrate of BAP1 [5]. However, BAP1 is part of essential cell cycle regulators [6] and probably associated with regulation of transcription [7]. BAP1 binds and deubiquitinates the transcriptional regulator host cell factor 1 (HCF-1), which interacts with histone-modifying complexes [8,9]. Together, these data indicate a complex mode of action for BAP1 involving different cellular pathways. It is even hypothesized that BAP1 effects can vary in different cell types and/or species [10]. BAP1 was shown to fulfill criteria of a genuine tumor suppressor gene, which presumably becomes apparent after a two-step inactivation according Knudson's two-hit model [4,9,11]: one allele of BAP1 being inactivated via inherited mutation (or monosomy of chromosome 3 [12]); and the remaining allele being lost by somatic BAP mutation(s) leading to biallelic inactivation [9,13].

Sporadic BAP1 mutations have been described in uveal melanoma, cutaneous melanoma and other melanocytic tumors, renal cell carcinoma and other cancers [9]. Besides the common sporadic BAP1 mutations, germline BAP1 mutations have been detected in families with a high incidence of MPM [14]. Individuals with heterozygous BAP1 germline mutations are affected by a newfound tumor predisposition syndrome characterized by very high risk of developing MPM, uveal melanoma (UV), cutaneous melanoma, atypical melanocytic benign neoplasms [15,16]

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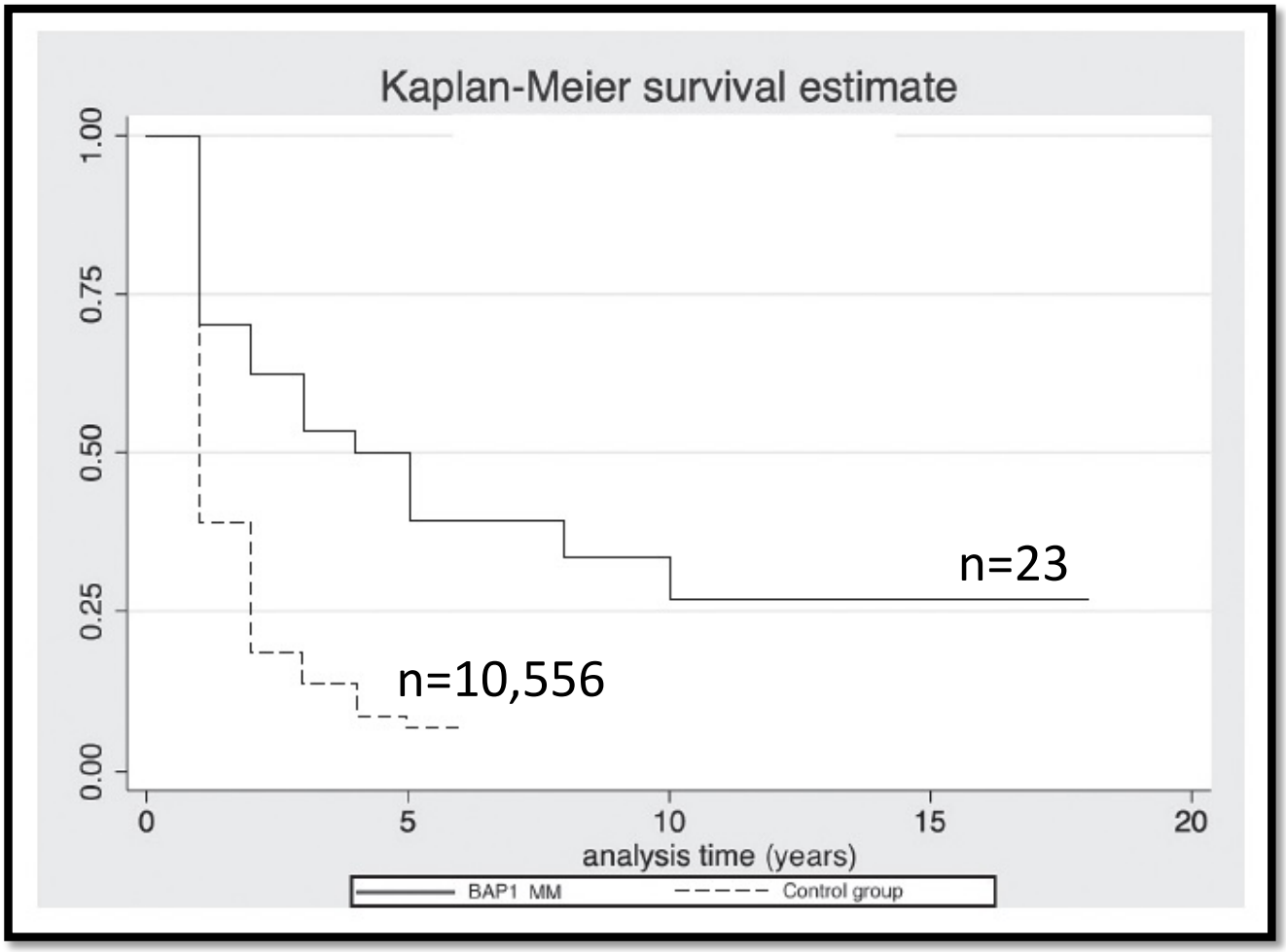
http://dx.doi.org/10.1016/j.lungcan.2014.10.017
0169-5002/

Rusch A, et al. Prevalence of BRCA-1 associated protein 1 germline mutation in sporadic malignant pleural mesothelioma cases. Lung Cancer. 2015 Jan;87(1):77-9.

MM Patients With BAP1 Mutations Live Longer



Baumann F et al. Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. Carcinogenesis. 2015 Jan;36(1):76-81.

BAP1 Mutations and MM

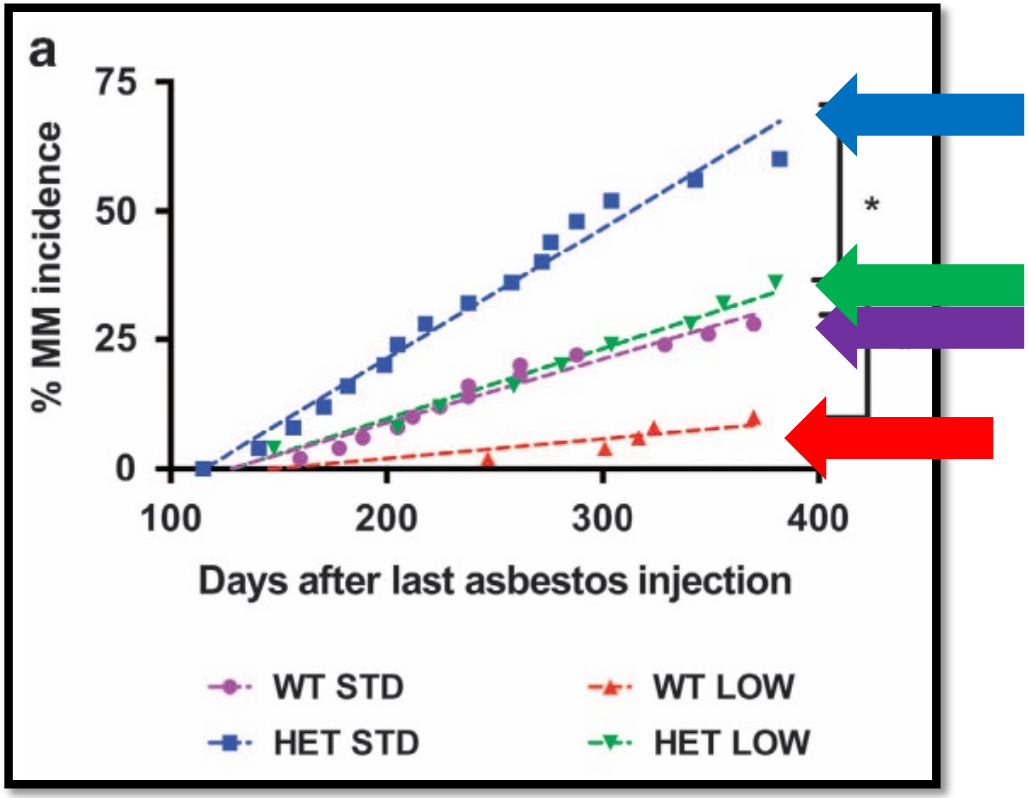
- BAP1 mutations predispose to familial MM
- BAP1 mutations less associated with sporadic MM
- MM patients with BAP1 mutations live longer than MM patients generally

- What about susceptibility to asbestos exposure?

BAP1 Knockout Mouse

Evidence for Susceptibility

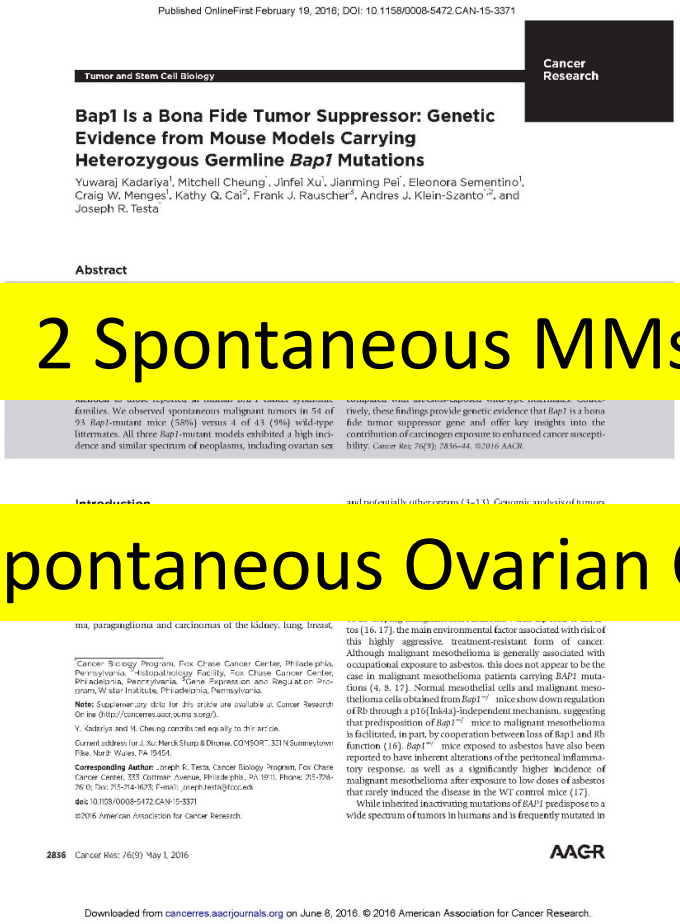
BAP1 Mutations Increase Asbestos-Induced Mesothelioma



Napolitano A, et al. Minimal asbestos exposure in germline BAP1 heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. Oncogene. 2015 Jun 29.

Evidence for Predisposition

Spontaneous Tumors in BAP1 Knockouts - No Asbestos



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Tumor and Stem Cell Biology

Cancer Research

Bap1 Is a Bona Fide Tumor Suppressor: Genetic Evidence from Mouse Models Carrying Heterozygous Germline *Bap1* Mutations

Yuwaraj Kadariya[1], Mitchell Cheung[1], Jinfei Xu[1], Jianming Pei[1], Eleonora Sementino[1], Craig W. Menges[1], Kathy Q. Cai[2], Frank J. Rauscher[3], Andres J. Klein-Szanto[1,2], and Joseph R. Testa[1]

Abstract

families. We observed spontaneous malignant tumors in 54 of 93 *Bap1*-mutant mice (58%) versus 4 of 43 (9%) wild-type littermates. All three *Bap1*-mutant models exhibited a high incidence and similar spectrum of neoplasms, including ovarian sex

...rely, these findings provide genetic evidence that *Bap1* is a bona fide tumor suppressor gene and offer key insights into the contribution of carcinogen exposure to enhanced cancer susceptibility. *Cancer Res; 76(9); 2836–44. ©2016 AACR.*

2 Spontaneous MMs

63% Spontaneous Ovarian Cancers

Introduction

...and potentially other organs (1–13). Genomic analysis of tumors

...ma, paraganglioma and carcinomas of the kidney, lung, breast,

...tos (16, 17), the main environmental factor associated with risk of this highly aggressive, treatment-resistant form of cancer. Although malignant mesothelioma is generally associated with occupational exposure to asbestos, this does not appear to be the case in malignant mesothelioma patients carrying *BAP1* mutations (4, 8, 17). Normal mesothelial cells and malignant mesothelioma cells obtained from *Bap1*$^{+/-}$ mice show down regulation of Rb through a p16(Ink4a)-independent mechanism, suggesting that predisposition of *Bap1*$^{+/-}$ mice to malignant mesothelioma is facilitated, in part, by cooperation between loss of Bap1 and Rb function (16). *Bap1*$^{+/-}$ mice exposed to asbestos have also been reported to have inherent alterations of the peritoneal inflammatory response, as well as a significantly higher incidence of malignant mesothelioma after exposure to low doses of asbestos that rarely induced the disease in the WT control mice (17).

While inherited inactivating mutations of *BAP1* predispose to a wide spectrum of tumors in humans and is frequently mutated in

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Y. Kadariya and M. Cheung contributed equally to this article.

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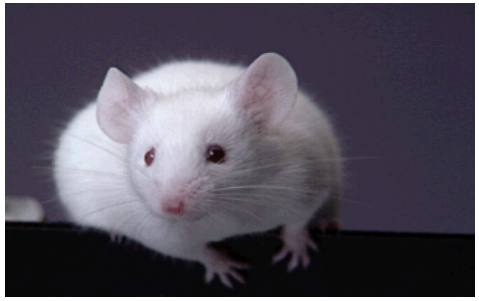
doi: 10.1158/0008-5472.CAN-15-3371

Do BAP1 Mutations Increase Susceptibility to Chrysotile-Induced MM?

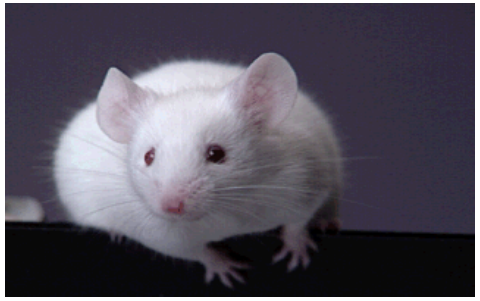
Amphibole Asbestos



BAP1 Mutant

↑ MM Susceptibility

Chrysotile Asbestos

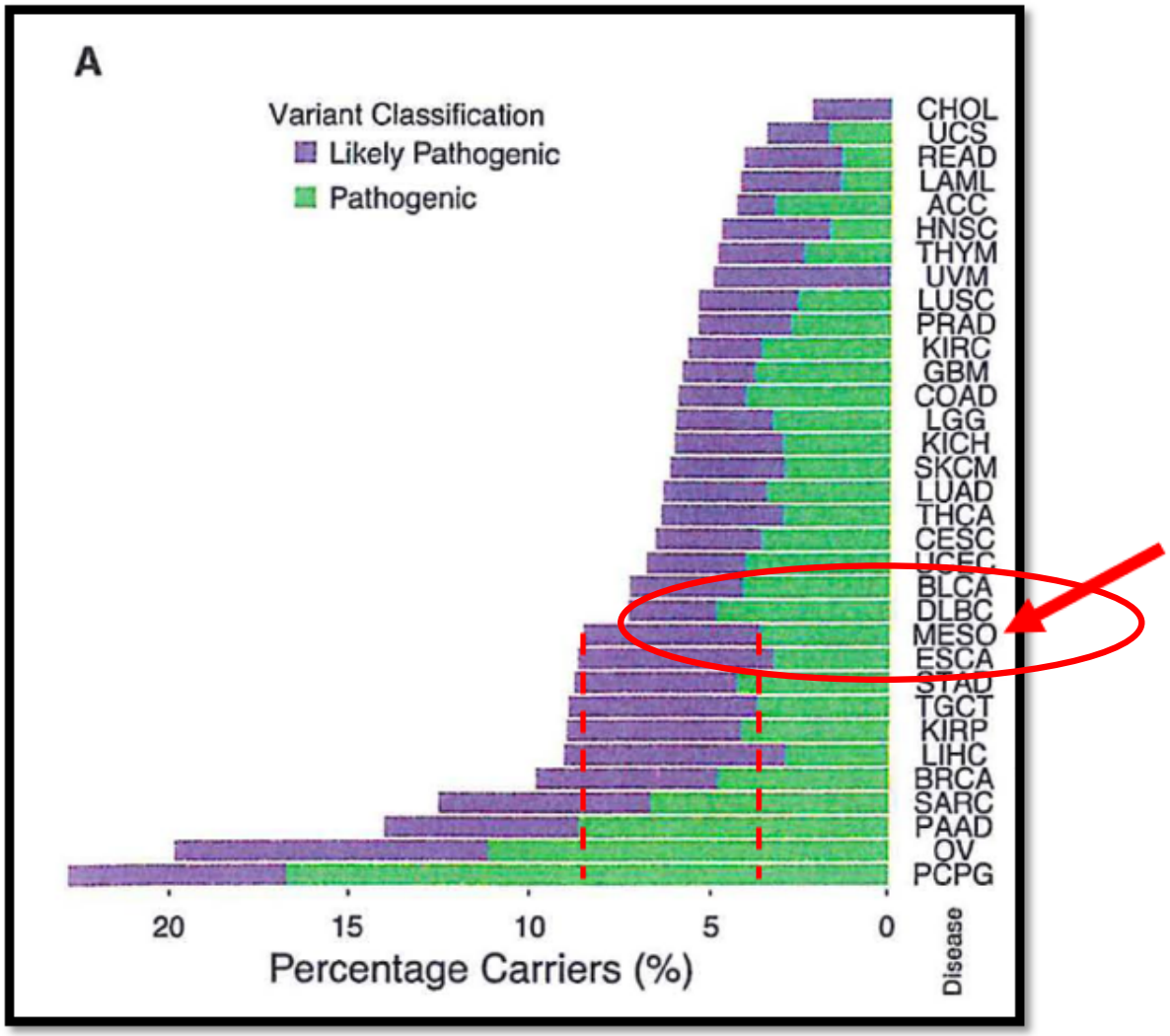


BAP1 Mutant

??? MM Susceptibilty

Other Mutations Linked to Mesothelioma

Mesothelioma (Like Other Cancers) Has Genetic Drivers



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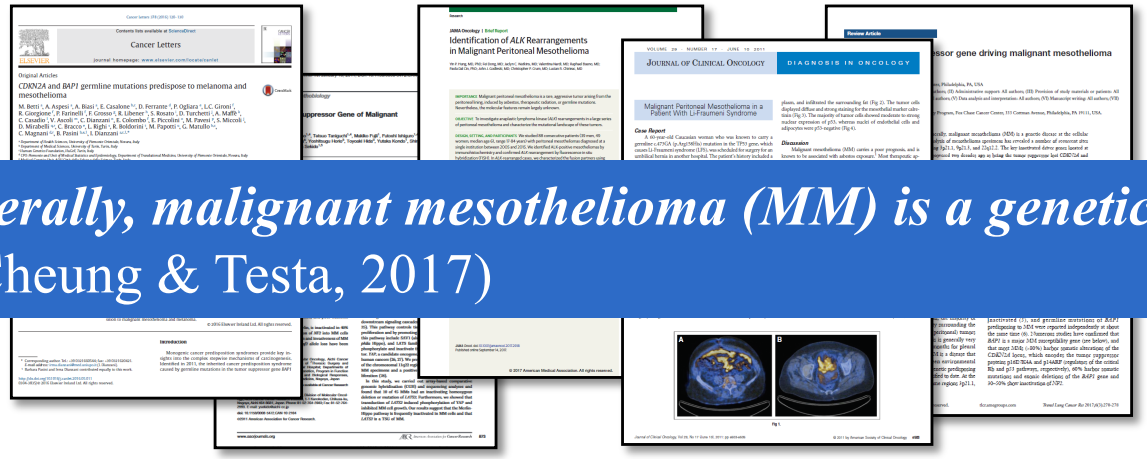
Mesothelioma is a Disease of the Genome

ALK: *"*We identified unique ALK rearrangements in a subset of patients with peritoneal mesothelioma, each lacking asbestos fibers..."

CDKN2A: *"*Our study suggests that CDKN2A, in addition to BAP1, could be involved in the melanoma and mesothelioma susceptibility, leading to the rare familial cancer syndromes."

TP53: "The mutated TP53 tumor suppression gene likely underlies the development of MM in this patient and is the cause of the familial syndrome."



"Like cancer generally, malignant mesothelioma (MM) is a genetic disease at the cellular level." (Cheung & Testa, 2017)

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Talc and Ovarian Cancer

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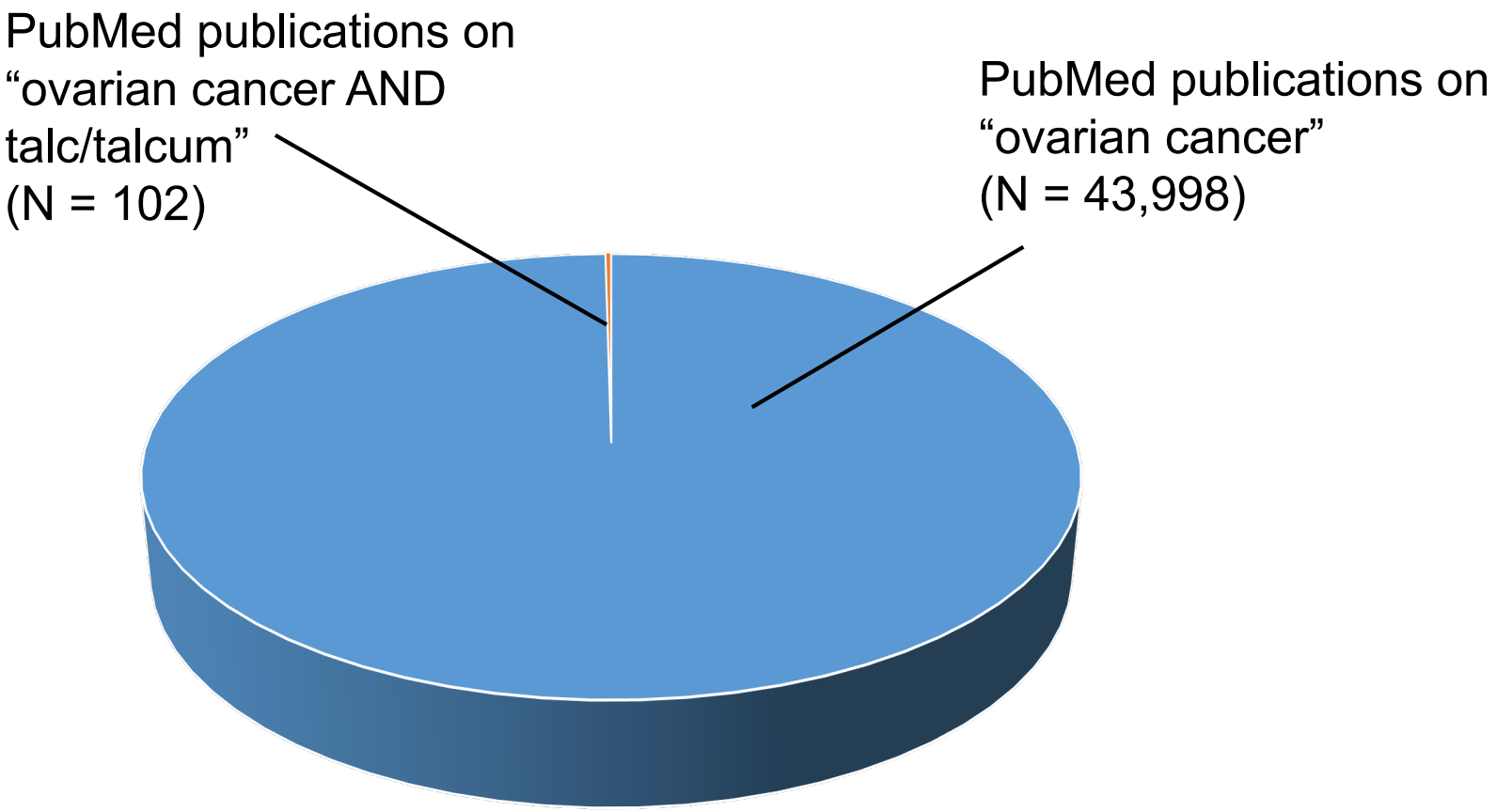
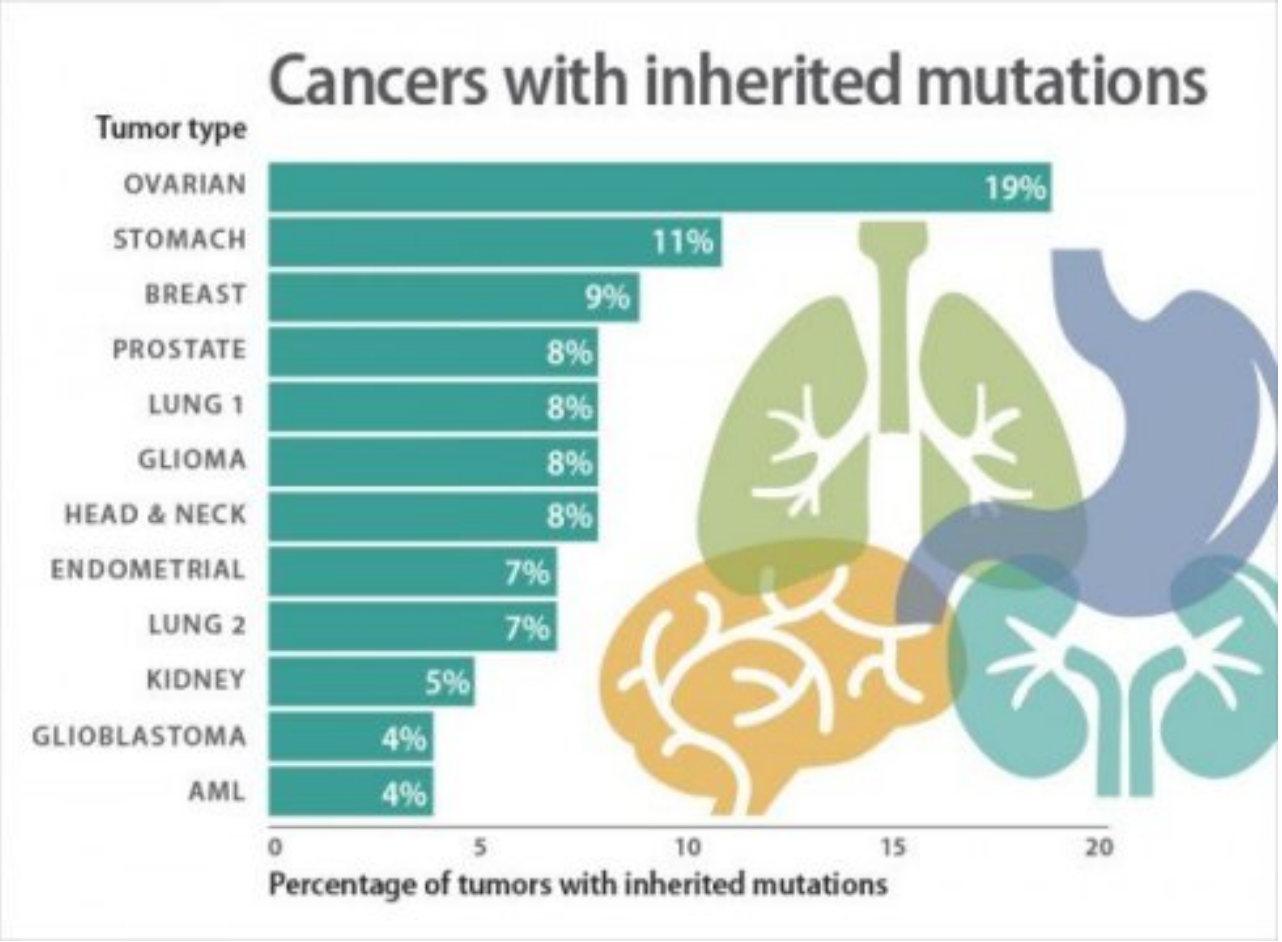
OC Cancer Researchers Are Not Studying Talc



Inherited Mutations Drive Ovarian Cancer



https://www.sciencedaily.com/releases/2015/12/151222084730.htm

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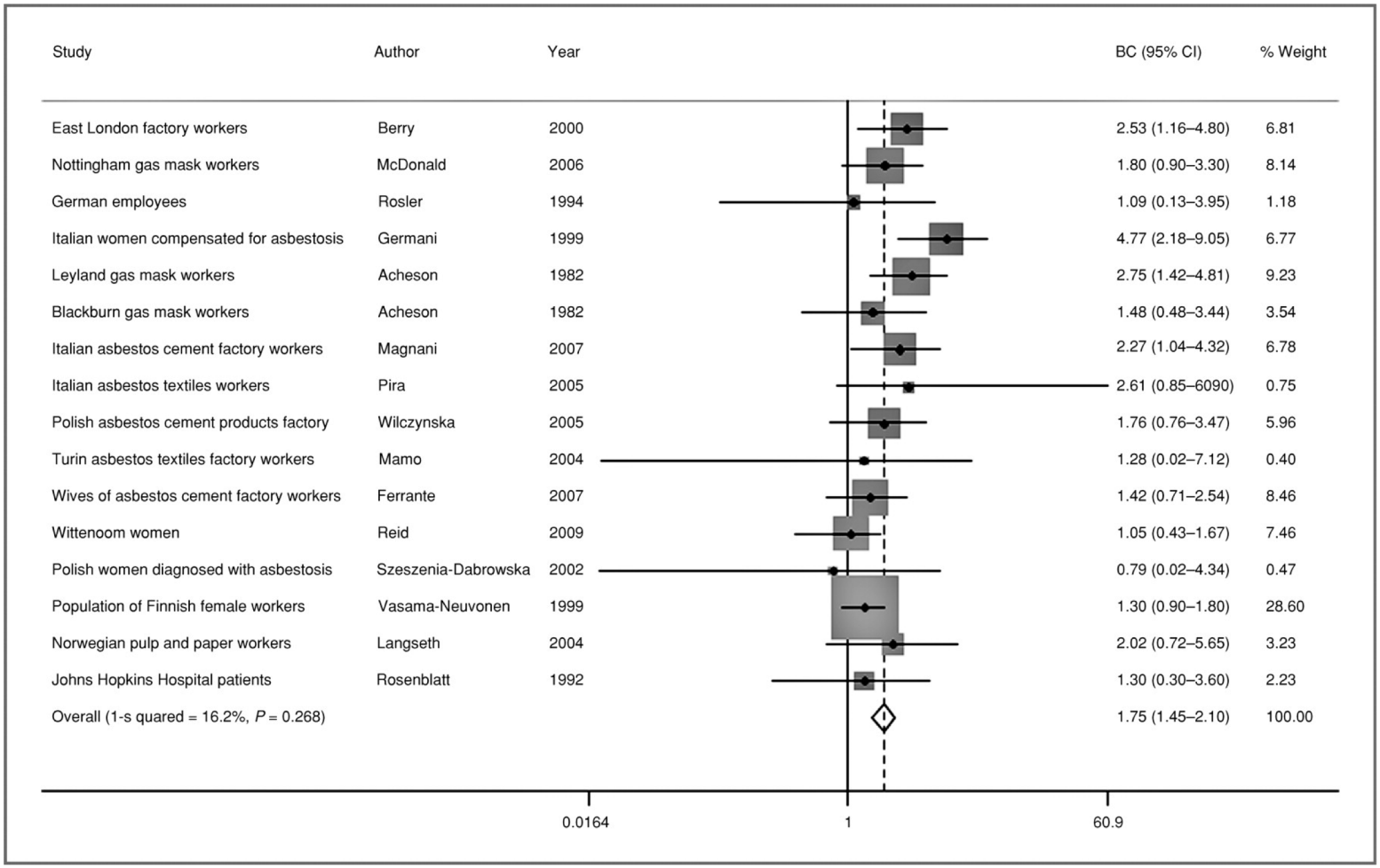
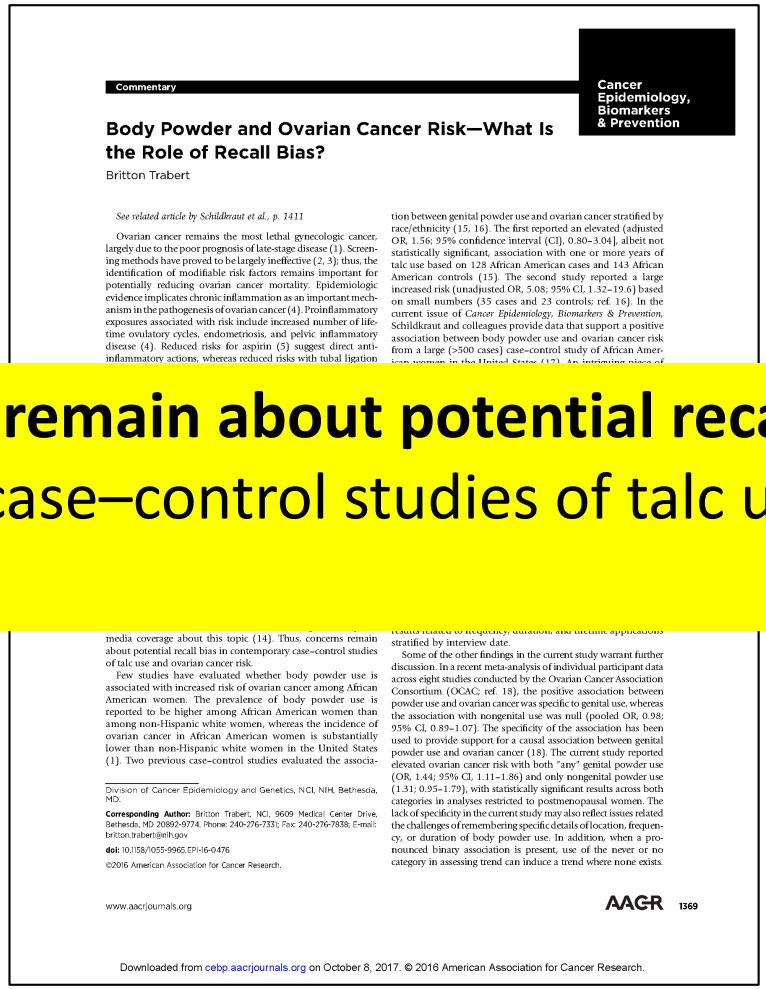
Epidemiological Studies on Talc and Ovarian Cancer



Case Control Studies Subject to Recall Bias



Commentary

Cancer Epidemiology, Biomarkers & Prevention

Body Powder and Ovarian Cancer Risk—What Is the Role of Recall Bias?

Britton Trabert

See related article by Schildkraut et al., p. 1411

Ovarian cancer remains the most lethal gynecologic cancer, largely due to the poor prognosis of late-stage disease (1). Screening methods have proved to be largely ineffective (2, 3); thus, the identification of modifiable risk factors remains important for potentially reducing ovarian cancer mortality. Epidemiologic evidence implicates chronic inflammation as an important mechanism in the pathogenesis of ovarian cancer (4). Proinflammatory exposures associated with risk include increased number of lifetime ovulatory cycles, endometriosis, and pelvic inflammatory disease (4). Reduced risks for aspirin (5) suggest direct anti-inflammatory actions, whereas reduced risks with tubal ligation

tion between genital powder use and ovarian cancer stratified by race/ethnicity (15, 16). The first reported an elevated (adjusted OR, 1.56; 95% confidence interval (CI), 0.80–3.04], albeit not statistically significant, association with one or more years of talc use based on 128 African American cases and 143 African American controls (15). The second study reported a large increased risk (unadjusted OR, 5.08; 95% CI, 1.32–19.6) based on small numbers (35 cases and 23 controls; ref. 16). In the current issue of *Cancer Epidemiology, Biomarkers & Prevention*, Schildkraut and colleagues provide data that support a positive association between body powder use and ovarian cancer risk from a large (>500 cases) case–control study of African Amer-

media coverage about this topic (14). Thus, concerns remain about potential recall bias in contemporary case–control studies of talc use and ovarian cancer risk.

Few studies have evaluated whether body powder use is associated with increased risk of ovarian cancer among African American women. The prevalence of body powder use is reported to be higher among African American women than among non-Hispanic white women, whereas the incidence of ovarian cancer in African American women is substantially lower than non-Hispanic white women in the United States (1). Two previous case–control studies evaluated the associa-

stratified by interview date.

Some of the other findings in the current study warrant further discussion. In a recent meta-analysis of individual participant data across eight studies conducted by the Ovarian Cancer Association Consortium (OCAC; ref. 18), the positive association between powder use and ovarian cancer was specific to genital use, whereas the association with nongenital use was null (pooled OR, 0.98; 95% CI, 0.89–1.07). The specificity of the association has been used to provide support for a causal association between genital powder use and ovarian cancer (18). The current study reported elevated ovarian cancer risk with both "any" genital powder use (OR, 1.44; 95% CI, 1.11–1.86) and only nongenital powder use (1.31; 0.95–1.79), with statistically significant results across both categories in analyses restricted to postmenopausal women. The lack of specificity in the current study may also reflect issues related the challenges of remembering specific details of location, frequency, or duration of body powder use. In addition, when a pronounced binary association is present, use of the never or no category in assessing trend can induce a trend where none exists.

Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD.

Corresponding Author: Britton Trabert, NCI, 9609 Medical Center Drive, Bethesda, MD 20892-9774. Phone: 240-276-7331; Fax: 240-276-7838; E-mail: britton.trabert@nih.gov

doi: 10.1158/1055-9965.EPI-16-0476

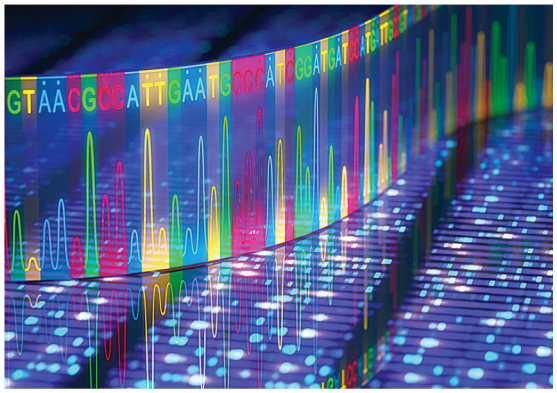
Thus, **concerns remain about potential recall bias** in contemporary case–control studies of talc use and ovarian cancer risk.

Trabert: Cancer Epidemiol Biomarkers Prev 2016;25:1369-1370.

How Do We Compare?

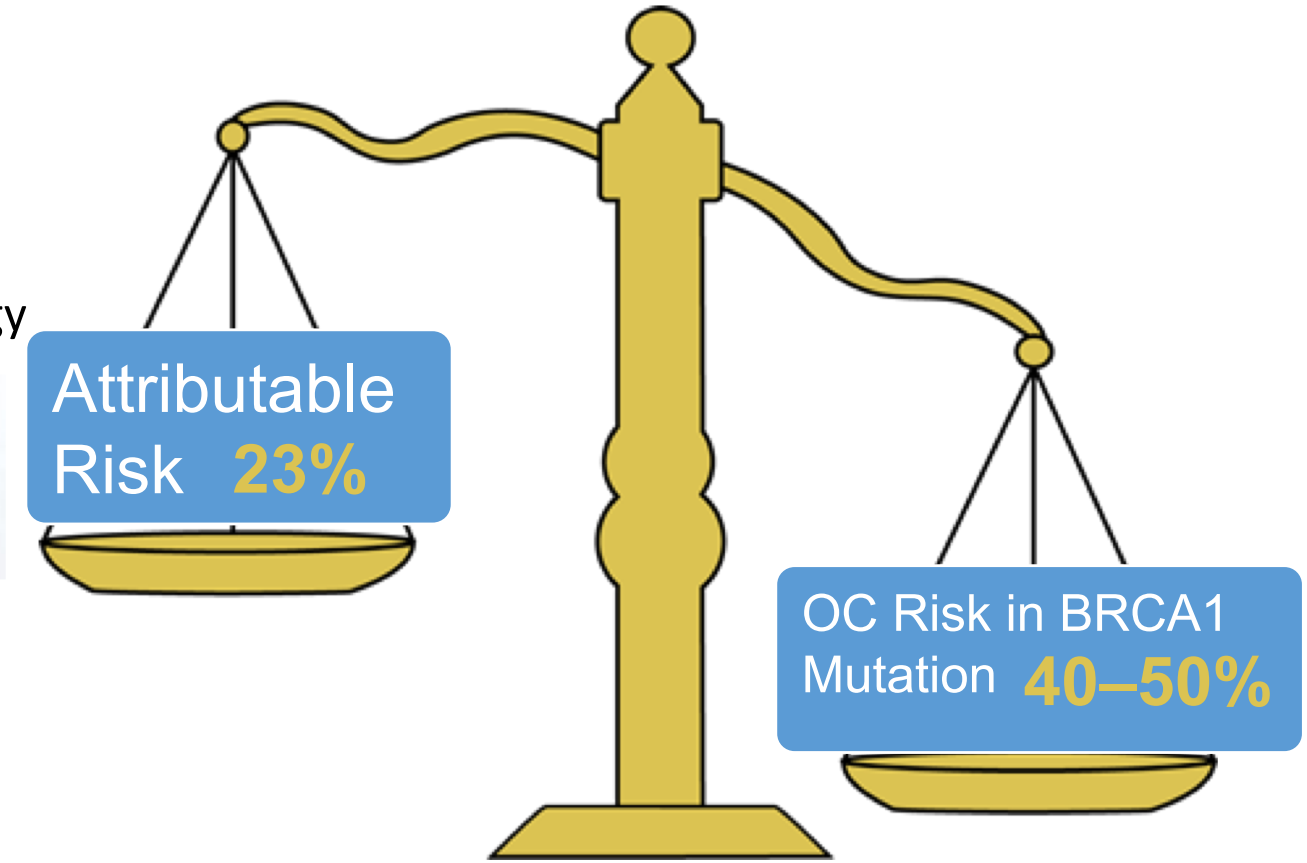
Black Box Epidemiology

Patient-Level Genomic Data

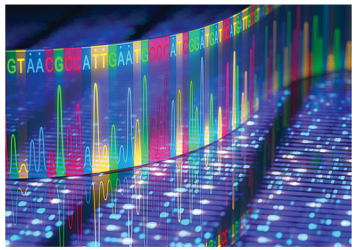


Compare role of Genetic Pattern to Attributable Risk

Black Box Epidemiology



Genetic Techniques



Genomics Technology Is Proven

Top Cancer Hospitals*

1. University of Texas M.D. Anderson Cancer Center
2. Memorial Sloan-Kettering Cancer Center
3. Johns Hopkins Hospital
4. Mayo Clinic
5. Dana-Farber/Brigham and Women's Cancer Center
6. Cleveland Clinic
7. Massachusetts General Hospital
8. University of Washington Medical Center
9. Ronald Regan UCLA Medical Center
10. Barnes-Jewish Hospital/Washington University
11. University of Maryland Medical Center
12. UCSF Medical Center
13. Duke University Medical Center
14. University of Michigan Hospitals and Health Centers
15. Stanford Hospital and Clinics
15. University of Chicago Medical Center
17. New York – Presbyterian University Hospital of Columbia and Cornell
18. Seidman Cancer Center at UH Case Medical
19. Hospital of the University of Pennsylvania
20. Thomas Jefferson University Hospital
21. University of Minnesota Center
22. Moffitt Cancer Center
23. City of Hope
23.University of Iowa Hospitals and Clinics
25. Ohio State University James Cancer Hospital
26. Wake Forest Baptist Medical Center
27. Northwestern Memorial Hospital
28. UPMC-University of Pittsburg Medical Center
29. Vanderbilt University Medical Center
30. NYU Langone Medical Center
31. Hackensack University Medical Center
32. Indiana University Health
33. Cedars-Sinai Medical Center
33. University of Colorado Hospital
35. Yale-New Haven Hospital
36. Shands at the University of Florida
37. University of Kansas Hospital
38. Methodist Hospital
39. Emory University Hospital
40. Nebraska Medical Center
41. University of Wisconsin Hospital and Clinics
42. Mount Sinai Medical Center
43. University of North Carolina Hospitals
43. USC Norris Cancer Hospital
45. Magee-Womens Hospital of UPMC
46. University of California, Davis Medical Center
47. Roswell Park Cancer Institute
48. Beth Israel Deaconess Medical Center
49. Robert Wood Johnson University Hospital
50. Fox Chase Cancer Center

Conclusions

Why Evaluate Genetics in a Toxic Tort Case?

1. May demonstrate that cancer was “predestined” in an individual plaintiff
2. Define genetic pathways that describe disease induction
3. May ultimately characterize exposures
4. Help to define subgroups where exposure is relevant
5. Help to define prognosis
6. Better equipped to respond to opponent’s case
7. **Makes the case about the individual – not a population**

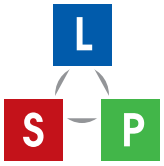
Update on Genomics in Mesothelioma Litigation

American Conference Institute

Chicago, Illinois

May 21, 2018

Kirk T. Hartley



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Writings Regarding Genomics In Toxic Tort Cases

Overview – Events and Articles - Genomics in Toxic Tort Litigation

- Workshop - Genetics in Civil Law (Washington DC May 2017)
  - http://www.a2lc.com/download-genetics-in-civil-law-conference-slides-2017
- Gary E. Marchant, *Genetic Data in Toxic Tort Litigation*, The Brief, Winter 2016, at 22
- Jennifer M. Champagne, *Genetic Testing and Testimony in Toxic Tort Litigation: Admissibility and Evaluation*, 13 N.C. J.L. & Tech. 1 (2011)(online @ http://scholarship.law.unc.edu/ncjolt/vol13/iss1/3)
- Gary E. Marchant, *Genetic Data in Toxic Tort Litigation*, 14 J. L. & Policy (2006)
  - http://brooklynworks.brooklaw.edu/jlp/vol14/iss1/2
  - https://papers.ssrn.com/sol3/papers.cfm?abstract_id=800044
- Fall 2018 – Marchant & Hartley – ABA text book on genetics in personal injury cases

Genomic Analysis Focuses on Individual Variability

- 2015 - a striking statement from an MD/PhD, and frequent expert in mesothelioma cases

- In an invited commentary, 44 Journal of International Journal of Epidemiology 1425-26 (2015), Dr. Suresh Moolgavkar wrote:
  - "large differences in susceptibility are determined by major gene defects or by events occurring in embryonic life that alter populations of critical cells…."

- Dr. Moolgavkar previously worked at a major cancer center, and also is an epidemiology focused MD/PhD at Exponent

- Dr. Moolgavkar also acknowledged that existing models for incidence and development of cancer do not account for individual variability

- Therefore, individual variability undercuts existing traditional epidemiology and other thinking that assumes "one size fits all"

Genomics in Particular Mesothelioma Cases

Evolution of Mesothelioma Cases With BAP1 Issues

- First BAP1 papers published in 2011
- BAP1 described at litigation conferences by Hartley, Dr. Brody and then others

- BAP1 entered litigation
- Andrea Huston – Kazan firm - associate
- 2014 declaration regarding five mesothelioma cases (listed below) with BAP1 genetic testing issues – for article with link to her declaration, see https://www.globaltort.com/2014/11/asbestos-litigation-goes-molecular-first-bap1-mutation-issues-reach-a-judge/
  - Ortwein v. CertainTeed Corp., et al., Alameda County Superior Court No. RG13701633
  - Perez v. ArvinMeritor, Inc., et al. , Alameda County Superior Court No. RG13689541
  - McCarthy v. Baltimore Aircoil Co., et al., Los Angeles County Superior Court No. BC464985
  - Bergstrom v. 84 Lumber, et al., Missouri Circuit Court (22nd Cir.) No. 1322-CC09325
  - Bernard v. Colgate-Palmolive Co., New York Supreme Ct., New York County, No. 107211/08

First Trial Regarding BAP1 - Holly Ortwein Case – 2016

- Mrs. Ortwein was 4th in her family to develop mesothelioma
- Possible low dose exposures included some intake home and other, related to a/c pipe)
- Kazan firm sought to block genetic testing; several briefs and hearings
- BAP1 testing was allowed – see article with link to order
  - https://www.globaltort.com/2015/01/asbestos-litigation-order-on-motion-to-compel-production-of-bodily-materials-to-test-for-a-germline-bap1-mutation/

- Case went to trial in January 2016 – Judge Seligman – Alameda County

- Mrs. Ortwein's lawyers (Satterley, Bosl, Huston) affirmatively raised her inherited BAP1 mutation, and argued disease can arise with lower doses – crocidolite at issue
  - Dr. Joseph Testa for plaintiff – lower dose can cause meso
  - Judge Seligman thought the issues interesting, and allowed jurors to submit questions to him, which he then asked after discussions with lawyers
  - Good questions were posed by jurors - see Schwartz and Hartley articlehttps://www.law360.com/articles/893614/jurors-in-toxic-tort-litigation-take-genetics-seriously
  - Case settled before testimony by defense expert (Dr. Feingold)

After Ortwein - Genetics in Other California Asbestos Cases

- Nolan Lamb – Contra Costa County
  - Brayton firm for 33 year old male with peritoneal mesothelioma
  - CertainTeed sought genetic testing, and plaintiff stipulated to allow
  - testing revealed BAP1 variant (mutation)
  - case was tried, two pathologists as experts
    - Dr. Sobonya for plaintiff
    - Dr. Feingold for defense
  - defense verdict – wisdom is verdict was based on other factors
- Cynthia Marshall – Alameda County
  - Kazan firm opposed BAP1 testing
  - Kazan firm published/presented abstract and posters regarding Marshall
  - Kazan firm argued there is “no evidence” BAP1 variant - by itself - will lead to mesothelioma
  - Defendant lost – Dr. Feingold as expert

More on Cynthia Marshall Case

- Per abstract from Kazan firm for its poster at iMig 2018

- "Results: The court ruled defendant would not be permitted to conduct genetic testing because: (1) defendant's expert did not show that a BAP1 mutation could cause cancer; (2) such a mutation would only make a person who had been exposed to carcinogens more likely to develop cancers, which is no defense; and (3) therefore genetic testing would not be directly relevant to any issue in the case. Consensus in the current medical literature indicates that "germline mutations in BAP1 may contribute to susceptibility to MM in asbestos exposed individuals." (Ohar, Jill A., et al. "Germline BAP1 mutational landscape of asbestos-exposed malignant mesothelioma patients with family history of cancer." Cancer Research 76.2 (2016): 206-215.)"

- May 2018 YouTube video by Steven Kazan regarding BAP1 posters at iMig meetings
  - https://www.youtube.com/watch?v=3iGWLA93n3s

More on Marshall …

iMig 2018 | 14th International Conference of the International Mesothelioma Interest Group

PP10.02: FURTHER UPDATE: IMPACT OF BAP1 MUTATION ON MESOTHELIOMA RISK AND IMPLICATIONS FOR MESOTHELIOMA LITIGATION

Steven Kazan[1], **Andrea Huston**[2]

[1]Kazan McClain Satterley & Greenwood, Oakland, UNITED STATES OF AMERICA, [2]Kazan, McClain, Satterley & Greenwood, Oakland, UNITED STATES OF AMERICA

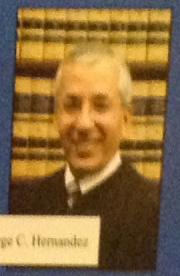
Background: Objectives: At iMig 2014 we presented on medical-ethical issues arising from the BAP1 mutation. (Abstract P1.061 and Poster P-149.) At iMig 2016 we discussed further developments in that case, including a defense claim that the plaintiff's mesothelioma was "caused" by a germline BAP1 mutation. (Abstract and Poster PP01.75.) In a recent case (Marshall v. Allied Fluid, et al., Alameda County Superior Court Case No. RG16843626), the Court found there was no basis for a claim that a BAP1 mutation causes cancer and disallowed genetic testing. Recent literature continues to conclude that BAP1 mutations do leave an individual increasingly vulnerable to carcinogens like asbestos, but are not BAP1 mutation are more vulnerable to oncogenesis of certain tumors after exposure to carcinogens, including asbestos exposure in the case of mesothelioma, the denial of requests for genetic testing was nonetheless appropriate because there is no credible expert evidence that a BAP1 mutation could have caused the cancer. The utility of BAP1 mutation as a defense to liability for mesothelioma is increasingly questionable, and will hopefully soon end.

Keywords: BAP1, mesothelioma, mutation, asbestos

- In response, cancer geneticist Joseph Testa, Ph.D., FACMG, contradicted Feingold's claims of causality. As Dr. Testa explained, "there is no scientific basis for [this] claim." [March 6, 2017 Declaration at 8:15-27; 12:22-24]:

> Attachments D). But inheritance of a mutation in one of the two copies of the *BAP1* gene is not, in and of itself, sufficient to be carcinogenic; i.e., it is not sufficient to act as a complete carcinogen to act as both tumor initiator and tumor promoter needed for tumor development.

- The Court agreed with Dr. Testa: "[Feingold] has not shown that a BAP1 mutation causes cancer and thus, that the presence of the mutation would be relevant to causation. Rather, as explained by [Dr. Testa], such a mutation only makes a person who has been exposed to carcinogens more likely to develop cancers – i.e., if present, it would make Plaintiff an "eggshell plaintiff." This is not relevant to causation or the amount of damages to which Plaintiff may be entitled." [Order, p. 1.]


Hon. George C. Hernandez

Methods

- Defendants' request for genetic testing was made in litigation, which led us to do a PubMed literature search on all peer-reviewed English language articles indexed between iMig 2016 and the present, with keyword searching for germline BAP1 and mesothelioma. Thirty-one articles were found.

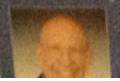
Results

- Since 2016, the majority of the literature continues to conclude that BAP1 mutations leave an individual increasingly vulnerable to carcinogens like asbestos. Consensus in the current medical literature indicates that "germline mutations in BAP1 may contribute to susceptibility to MM in asbestos exposed individuals" and "the high incidence of tumours associated with environmental stressors, such as mesothelioma (with asbestos) . . . highlights BAP1 as a critical player in the interaction of genes with environment."

Conclusion

- It is refreshing when Courts understand and appropriately rely on good science when making important decisions. In this case, current literature strongly supported the Court's conclusion that while individuals with a germline BAP1 mutation are more vulnerable to oncogenesis of certain tumors after exposure to carcinogens, including asbestos exposure in the case of mesothelioma, the denial of requests for genetic testing was nonetheless appropriate because there is no credible expert evidence that a BAP1 mutation could have caused the cancer. The utility of BAP1 mutation as a defense to liability for mesothelioma is increasingly questionable, and will hopefully soon end.

References
1 Kazan and Kin. Hippocrates and BAP1 Genetic Testing in Mesothelioma Litigation. Abstract P1.08-1. iMig 2014; p. 30
2 Kazan, Update: Recent Studies Examining Impact of BAP1 Mutation on Mesothelioma Risk and Implications for Mesothelioma Litigation. Abstract #P01-15. iMig 2016
3 Bhat, et al. Germline BAP1 mutational landscape of asbestos-exposed malignant mesothelioma patients with family history of cancer. Cancer Research 76.7 (2016): 204-213.
4 Artesio, Italia.. Genes versus Environment: cytoplasmic BAP1 determines the basic response to environmental stressors in mesothelioma. Cell Death Dis. (2017 Jun); 8(6): e2907


Steven Kazan,
Founding,

KAZAN, McCLAIN

After Ortwein – Non-California Examples of Mesothelioma Cases With Genetic Issues

- Following are non-California examples, not a complete list

- Blackford Cleeton – Richland County, IL – Simmons Hanley case
  - young woman (mid-30s) with melanoma and mesothelioma
  - take home exposure claimed via father who worked at refinery
  - variant (mutation) in CDKN2A gene
  - variant found through non-litigation testing

- Lanzo – Middlesex County, NJ (Judge Viscomi)
  - BAP1 genetic testing allowed

- Also in Middlesex County, NJ, one defendant sought genetic testing in two mesothelioma cases (Johnson and Lashley)
  - some other defendants joined formally or less formally
  - motions may be mooted by dismissals – ongoing as of today

- Bailey – Lawrence County, TN
  - genetic testing allowed despite plaintiff's objections

Examples of Plaintiffs Using Genomics In Toxic Tort Cases

Plaintiffs Successfully Used Genetics Against Defendants in Other Mass Tort Litigation

- **<u>Tumors in one year in Actos bladder cancer MDL - epigenetics</u>**
- Based on testimony from a world class UK researcher, a federsl MDL judge denied defense Daubert challenges
- Judge concluded a jury could plausibly find the drug at issue could cause bladder cancer in a year or less due to its epigenetic effects of drug for cells in tissue lining the bladder
  - *2013 U.S. Dist. LEXIS 179235*
- Not long thereafter, a $2+ billion settlement

- **<u>Molecular/genetic cancer explanation allowed in *Milward* in benzene litigation</u>**:
- Despite extensive "no cause" epidemiology, experts for plaintiff provided a molecular science explanation for a blood cancer allegedly caused by intake of benzene
- Despite amicus briefs from every major defense group, SCOTUS denied certiorari
- Today, some benzene defendants settle some cases that involve particular molecular signatures linked to benzene and blood cancers
- In other cases, benzene defendants use genetics to defeat cancer cases

Plaintiff Use of Genetic Testing in 2016
Brayton Purcell Lung Cancer Case

- Brayton firm – state and federal opinions in lung cancer case versus tobacco defendants

- *Pooshs v. Philip Morris USA, Inc.*, 51 Cal. 4th 788, 123 Cal. Rptr. 3d 578, 250 P.3d 181 (2011)(ruling on statute of limitations for multiple diseases)

- *Pooshs v. Philip Morris*, 2016 U.S. Dist. LEXIS 27240; 2016 WL 772405 – ruling on trial objections
- Interesting uses of genetics
  - Defendants first raised a genetic issue
  - Plaintiff used genetic testing to knock down the the defense arguments
  - See quotes on next slide

More – Plaintiff Use of Genetic Testing in Lung Cancer Case

- Quotes from lung cancer case v. tobacco defendants, *Pooshs v. Philip Morris*, 2016 U.S. Dist. LEXIS 27240

- "In his initial *Rule 26* expert report, Dr. Chirieac opined that plaintiff "developed a lung cancer induced by an activating mechanism in the epidermal growth factor receptor (EGFR) gene . . . that is unrelated to smoking." He provided several reasons to support his opinion that plaintiff has an EGFR-mutated cancer — e.g., good response to Tarceva (therapy most [*6] useful for EGFR-mutated cancers); very long survival since diagnosis; pathology showing "minimally invasive" adenocarcenoma with predominantly lepidic (BAC) and papillary features (typical of EGFR-mutated cancers); family history of cancer; and long period between cessation of smoking and cancer diagnosis. He also stated that "[a]dditional pathology may become available" — including tissue samples of the right adrenal gland — and that he "reserve[d] the right to review those slides and supplement this report."

- "As set forth by plaintiff in the opposition to RJR's Motion in Limine No. 1 (Doc. 280), plaintiff's counsel ordered further pathology testing in May 2012. The report indicated that the testing had detected no EGFR mutation, was negative for a rearrangement involving the ALK gene by FISH, and no KRAS mutations. On May 30, 2012, Dr. Barry Horn, plaintiff's disclosed pulmonology expert, submitted a supplemental report in which he discussed the results of the May 2012 testing, and noted that Dr. Hammar had indicated he disagreed with Dr. Chirieac's opinion."

- Dr. Chirieac stated that after he had reviewed the tests ordered by plaintiff's counsel … he ordered a more comprehensive test for [*8] detecting EGFR mutations by Sanger gene sequencing, and stated that those tests were also negative. He opined that "[i]n light of these test results, Mrs. Pooshs most likely does not have a known activating EGFR mutation."

Plaintiff Firms Promoting BAP1 Susceptibility to Mesotheliomas

- Several other cancers in the BAP1 syndrome – kidney cancer is now included with relative certainty – e.g. http://www.cancerindex.org/geneweb/BAP1.htm

- Web sites associated with plaintiff firms have many pages about genetic susceptibility to mesothelioma and other cancers, including ovarian cancer
  - https://www.asbestos.com/mesothelioma/genetic-factors.php
  - https://www.mesotheliomaguide.com/treatment/cure/genetic-testing/
  - http://www.mesotheliomafromnavy.com/blog/marjorie-zauderer-of-meso-foundation-receives-dod-grant/
  - http://mesothelioma-lawfirmtk.blogspot.com/2016/05/scientists-say-bap1-loss-may-be-gain.html
  - http://www.landryswarr.com/new-tests-for-meso-show-promise/

ToxicoGenomica – Expert Testimony on Genetics in Mesothelioma and Other Cases

Five Cases - Expert Testimony on Genetics - ToxicoGenomica.com

Examples of expert reports and testimony by Len van Zyl, Ph.D. - ArrayXpress

Plaintiff	Defendant	Age at diagnosis	Cancer	Toxicant
Mr. Cacoilo	Sherwin-Williams *et al.*	24	AML	benzene
Ms. Blackford-Cleeton	Marathon Oil *et al*.	32	Mesothelioma/ Melanoma	asbestos
Mr. Leach	BP *et al.*	58	AML	benzene
Mrs. Guzman	Exxon Mobil *et al.*	28	papillary thyroid cancer	α-radium ($^{226}Ra/^{228}Ra$)
Mr. Harvey	Sunoco *et al.*	34	AML	benzene

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ToxicoGenomica

1) is a multidisciplinary group of scientists and lawyers
2) offering genomics & systems biology services
3) including gene sequencing, and
4) evaluation of other objective biomarkers, such as studies of gene expression in persons with cancer

Blackford- Cleeton – Mesothelioma – CDKN2A Mutation

- Southern Illinois (Richland County) case involving a mid-30s woman with melanoma followed by mesothelioma
- Father worked at oil refinery
  - Issues regarding asbestos cement pipe
- Non-litigation genetic testing showed an inherited variant (mutation) in CDKN2A gene
  - gene provides instructions for a protein involved in repairing double strand breaks
  - Double strand breaks arise from tanning and smoking
  - Moolgavkar now has written that epidemiology incorrectly told us smoking is not involved in mesothelioma,
  - He now believes smoking is involved in a very small number of mesotheliomas – see 2017 Testa treatise on mesothelioma
- Dr. Len van Zyl (Ph.D.) testified for defense regarding pathways to develop mesothelioma regardless of asbestos exposure, if any
- Case settled
- Blackford-Cleeton case provides an example of why testing only for BAP1 is less informative

Beyond BAP1 - Mesothelioma and Additional Genes

- BAP1 is only one of 15-25 well known tumor suppressor genes;
  - using *in silico* analysis, some researchers assess over 1,200 genes as involved in tumor suppression
  - *See* TSGene database at Vanderbilt

- BAP1 discoverers (Carbone, Testa et al. 2011) think other genes also are factors

- "Our results provide the first demonstration that genetics influences the risk of mesothelioma, a cancer linked to mineral fiber carcinogenesis. As observed for *BRCA1* and *BRCA2*, which account for only some hereditary breast carcinomas, it appears likely that in addition to *BAP1*, more genes will be found associated with elevated risk of mesothelioma. Indeed, among our 26 sporadic mesotheliomas …
  - Testa, J.R., M. Cheung, J. Pei, et al (2011) Germline *BAP1* mutations predispose to malignant mesothelioma. *Nat Genet* 43:1022-1025.

More Beyond BAP1 - Mesothelioma and Additional Genes

- Variants (mutations) in multiple genes increase cancer risks
- Multi-gene genetic tests are increasing
  - "BROCA" gene panel test - Mary Claire King – UW - ongoing studies looking for BRCA genes, BAP1 and 38 other genes
- Liquid biopsy test (infra) results will be based on genomic analysis of DNA
- Precision medicine therapies are being applied after genomic testing of tumors and blood
- ToxicoGenomica - gene panels will focus on genes related to diseases at issues in litigation, such as mesotheliomas and ovarian cancers

Big Picture Issues and Projections Regarding Mesothelioma Causation

Big Picture Mesothelioma Issues, and Role of Genomics

- Per SEER, mesotheliomas remain high in US
  - Jorge Sirgo – Nathan Inc. (formerly Gnars)
  - https://www.nathaninc.com/sirgo-reviews-updated-seer-cancer-statistics/
- Mesotheliomas rising outside US
  - Jessica B. Horewitz, PhD., & Kirk T. Hartley, *A Global View of Mesotheliomas and Asbestos Litigation: Both Are Many Years Away from Peaking When Looking Outside the US (*summarizes work by Peto et al*)*
  - http://gnarusllc.com/wp-content/uploads/2016/08/Commentary.pdf
- Assertions regarding mesothelioma causation in Bestwall (Georgia-Pacific) chapter 11 case
- Bates White projections/arguments regarding future mesotheliomas
- Dr. Bertram Price/KCIC paper regarding current and future mesothelioma causation

Bestwall – Challenging Mesothelioma Causation

Bestwall/Georgia Pacific chapter 11 "informational brief" filed by Bestwall/Georgia Pacific on 11/2/17.
https://www.scribd.com/document/379701100/Bestwall-Georgia-Pacific-chapter-11-Doc-12c-Informational-Brief-as-Filed

Bestwall's statements include the following statements at:

Footnote 35: "Notably, Bestwall has faced a disproportionately large and growing number of female mesothelioma cases in recent years. From 2005 to 2016, the annual number of mesothelioma cases filed by female plaintiffs against Bestwall doubled. Because recent studies show that the vast majority of female mesotheliomas are idiopathic (i.e. not connected to any particular cause or exposure), these cases are far less likely to represent any valid claims that can be attributed to Bestwall. Moreover, women during and prior to the mid-1970s (when Bestwall's asbestos-containing products were last sold) were unlikely to have had occupational exposures in heavy industries and shipping. These cases often involve questionable product identification and exposure claims premised on household do-it-yourself projects. These dated, private, at-home exposure scenarios are particularly susceptible to questionable product-naming claims." (citing to "Michele Carbone, *et al.*, *Malignant Mesothelioma: Facts, Myths and Hypotheses*, 227(1) J. CELL. PHYSIOL. 44, 44 (2012).)

Footnote 34 - "A recent analysis of U.S. population data reports that the spontaneous or background mesothelioma rate is at least 27%. Bertram Price & Adam Ware, *Time Trend of Mesothelioma Incidence in the United States and Projection of Future Cases: an Update Based on SEER Data for 1973 Through 2005*, 39(7) CRIT. REV. TOXICOL. 576, 587 (2009)."

Mesothelioma Projections From Statisticians

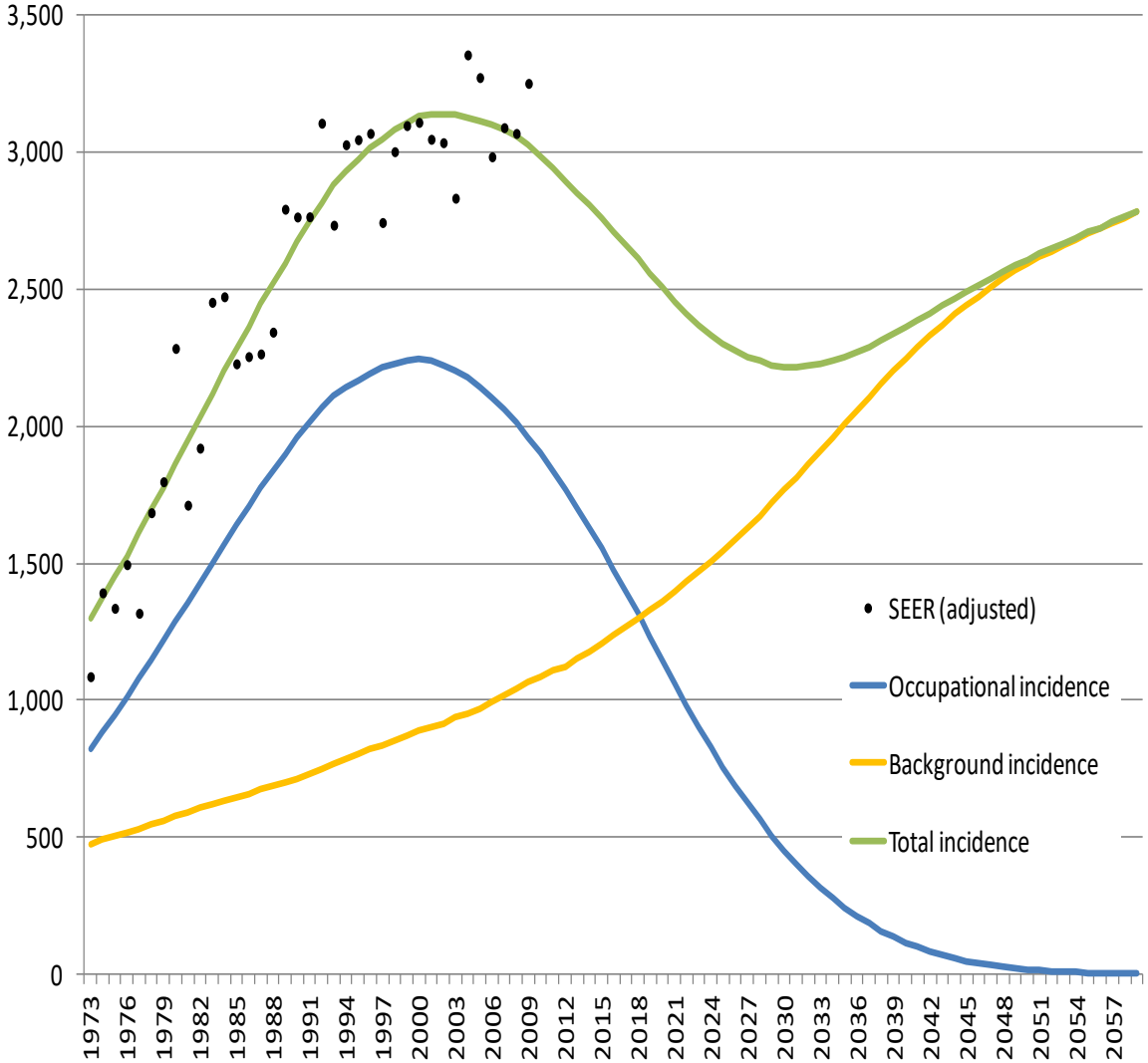
BATES WHITE
ECONOMIC CONSULTING

By the numbers: the future of mesothelioma in America

Jorge Gallardo-Garcia, PhD

March 18, 2014

In future years, the portion of incidence not attributable to asbestos exposure will continue to grow



Bertram Price, PhD – 2018 Article Regarding Mesothelioma Causation

- Recent article for KCIC by Bertram Price, PhD,
- "Mesothelioma: The Long Tail of Asbestos Personal Injury Litigation in the U.S."
  - https://www.kcic.com/trending/feed/the-proof-is-in-the-data-asbestos-isn-t-the-only-cause-of-mesothelioma/.
- As explained by KCIC, Dr. Price's new paper is a next step after prior papers:
  - "Time trend of mesothelioma incidence in the United States and projection of future cases: An update based on SEER data for 1973-2005" published in Critical Reviews in Toxicology (2009)
  - An update of projections he published in the American Journal of Epidemiology in 1997 and again in 2004."
- Article addresses some mesothelioma causation claims/arguments
- Article seem to put notable pressure on defense lawyers to use genetics and other aspects of science to defeat mesothelioma claims

More - Dr. Price

- **Key Points from section 1.0 - Abstract**

- "A long tail of asbestos personal injury litigation is looming that is fueled by mesothelioma cases that are not caused by asbestos.[1]

- These mesotheliomas are background cases of the disease, i.e., mesothelioma where the disease is a result of spontaneous tumor formation and is not a consequence of asbestos exposure or exposure to any other known risk factor for mesothelioma.

- In this update of my analysis and projections of future mesothelioma cases published in 2009,[2] the results indicate that during the five-year period from 2012 through 2016, an estimated 55% of all mesothelioma medical cases diagnosed in the U.S. were background cases; 37% of male mesotheliomas were background cases; and approximately 99% of all female mesothelioma cases were background cases.[3, 4]

- In approximately 20 years, starting around 2040, most, if not all, mesothelioma cases will be background cases.

- Leading up to and after 2040, there will be between 1,500 and 1,600 mesothelioma cases per year, virtually all background cases.[5]"

More Bertram Price/KCIC –

- Dr. Price's assertions are based on:
  - his interpretation of epidemiologic studies
  - Citation to Vogelstein/Tomasetti articles on "bad luck and cancer,"
    - "Bad Luck" papers focused on "random" mutations and rate of stem cell division and replication
- Defense-side lawyers cited to the first "bad luck" paper as perhaps providing a line of defense
  - http://www.ettdefenseinsight.com/2015/01/recent-science-article-a-potential-game-changer-for-arguing-medical-causation-in-cancer-cases-stem-cell-division-and-bad-luck/

Bert Price/KCIC – Challenging Mesothelioma Causation

- Dr. Charles Bates also cites to the "bad luck" articles when pressed to explain some of his mesothelioma projections

- "Bad luck" papers are interesting, but as the authors have said:

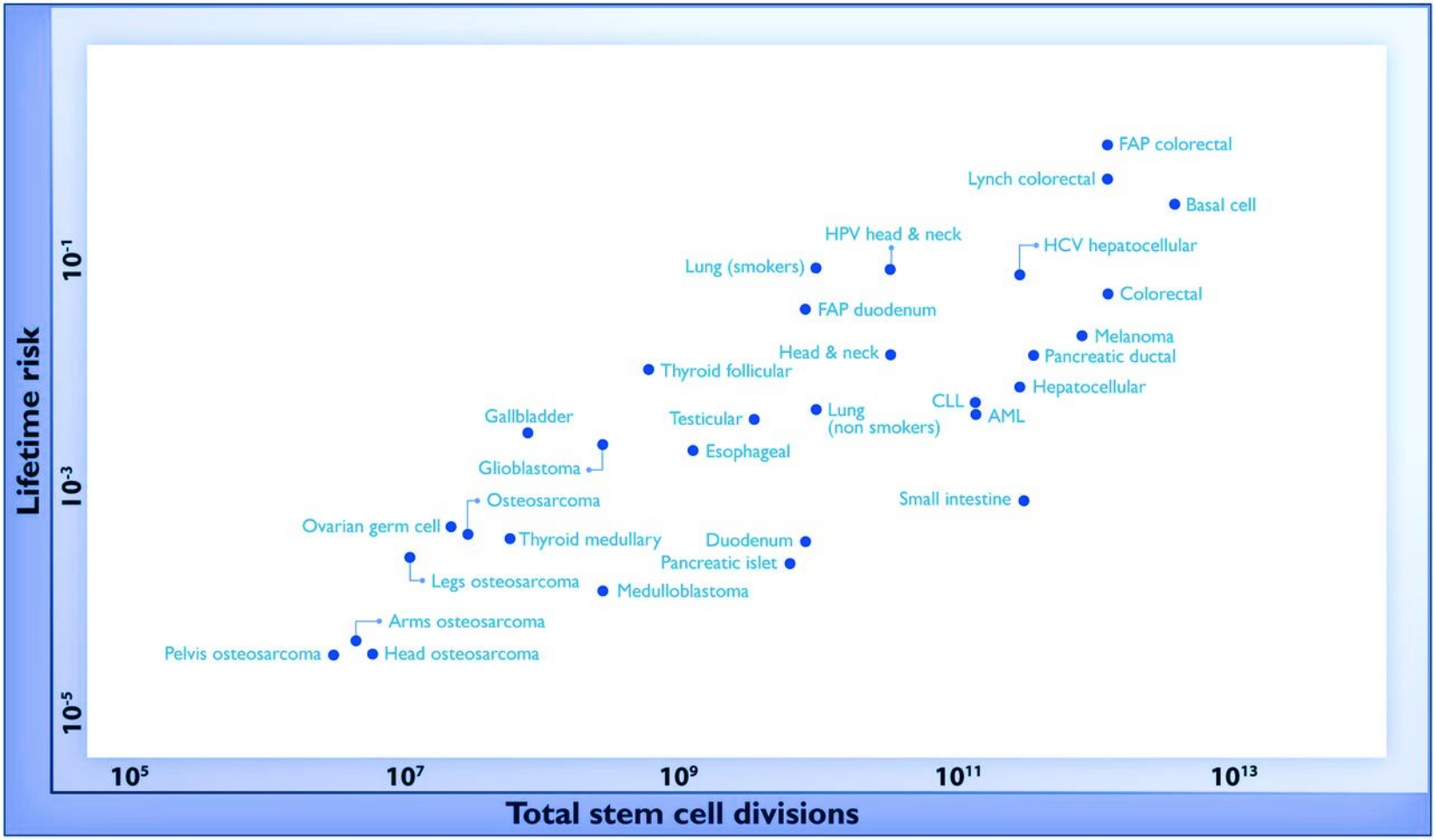
  - "This Bad Luck theory suggests that R[andom] mutations play a major role in cancer, but the correlation they found did not allow to measure how large that role is in any specific cancer type or in cancer overall."

  - "As with all scientific research, it will take time to consolidate (or disprove) the Bad Luck theory."

  - https://www.cristiantomasetti.com/bad-luck-theory/

- Therefore, genomic analysis is needed to understand a specific cancer in a specific person

Fig. 1 The relationship between the number of stem cell divisions in the lifetime of a given tissue and the lifetime risk of cancer in that tissue.



FAP = Familial Adenomatous Polyposis ◇ HCV = Hepatitis C virus ◇ HPV = Human papillomavirus ◇ CLL = Chronic lymphocytic leukemia ◇ AML = Acute myeloid leukemia

C Tomasetti, and B Vogelstein Science 2015;347:78-81

Improving Asbestos Quantification: What Can Be Done?

Jorge E. Sirgo, Gnarus Advisors, LLC

Casualty Loss Reserve Seminar

September 10, 2015

Sources of Disparity

- Context of Nicholson et. al. (1982) forecast and SEER data
  - SEER represents an estimate of diagnoses from all causes
  - Nicholson et. al. (1982) an estimate of deaths from those occupationally exposed to asbestos
- Outdated estimates of mortality
  - Nicholson et. al. (1982) uses mortality from 1975-1979
  - Life expectancy has increased
  - Use of static versus dynamic estimate
- Exposure level assumptions
  - Nicholson et. al. (1982) assumes reduced levels as "adoption for control measures" in 1972-1979

2016 – Objective Data from Roggli and Colleagues Regarding Mesothelioma and Asbestos Fiber Burden Data

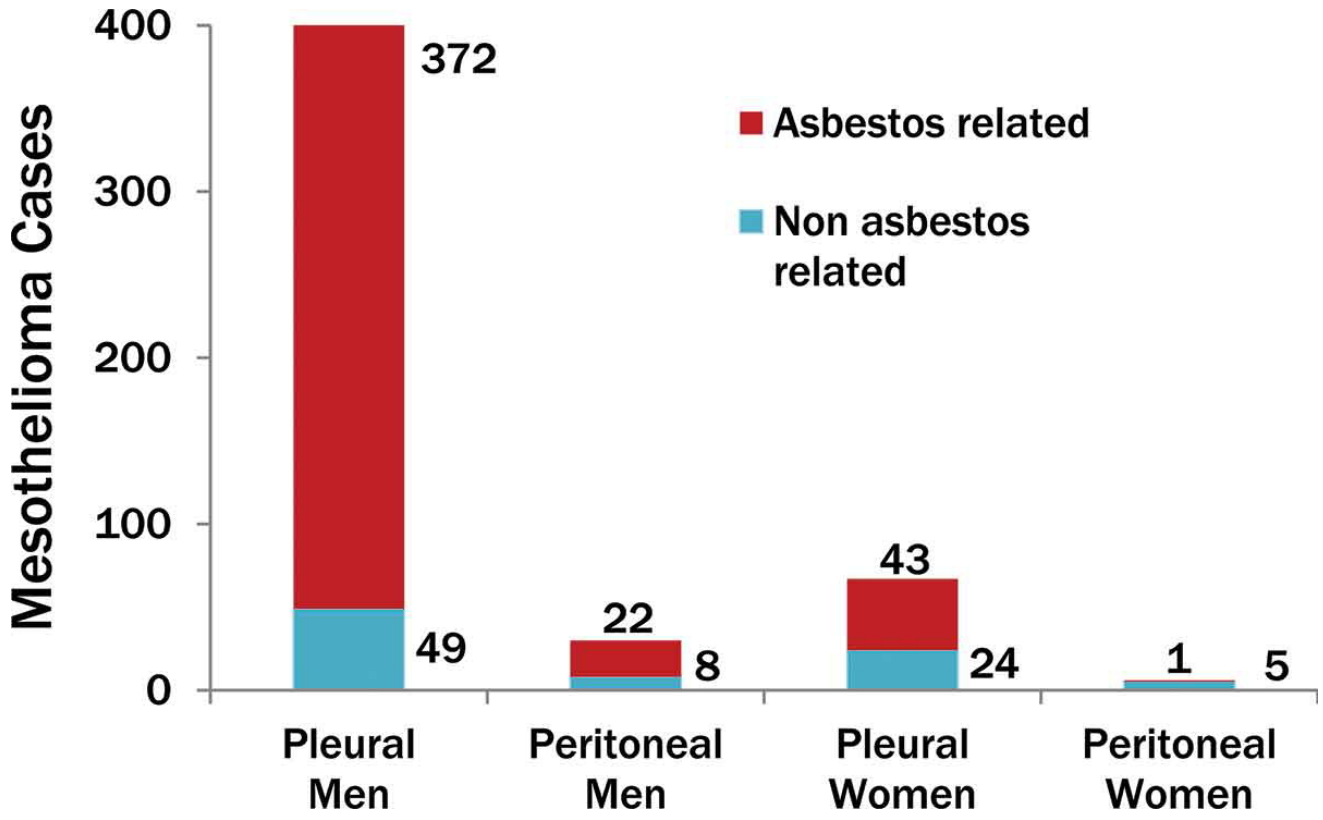


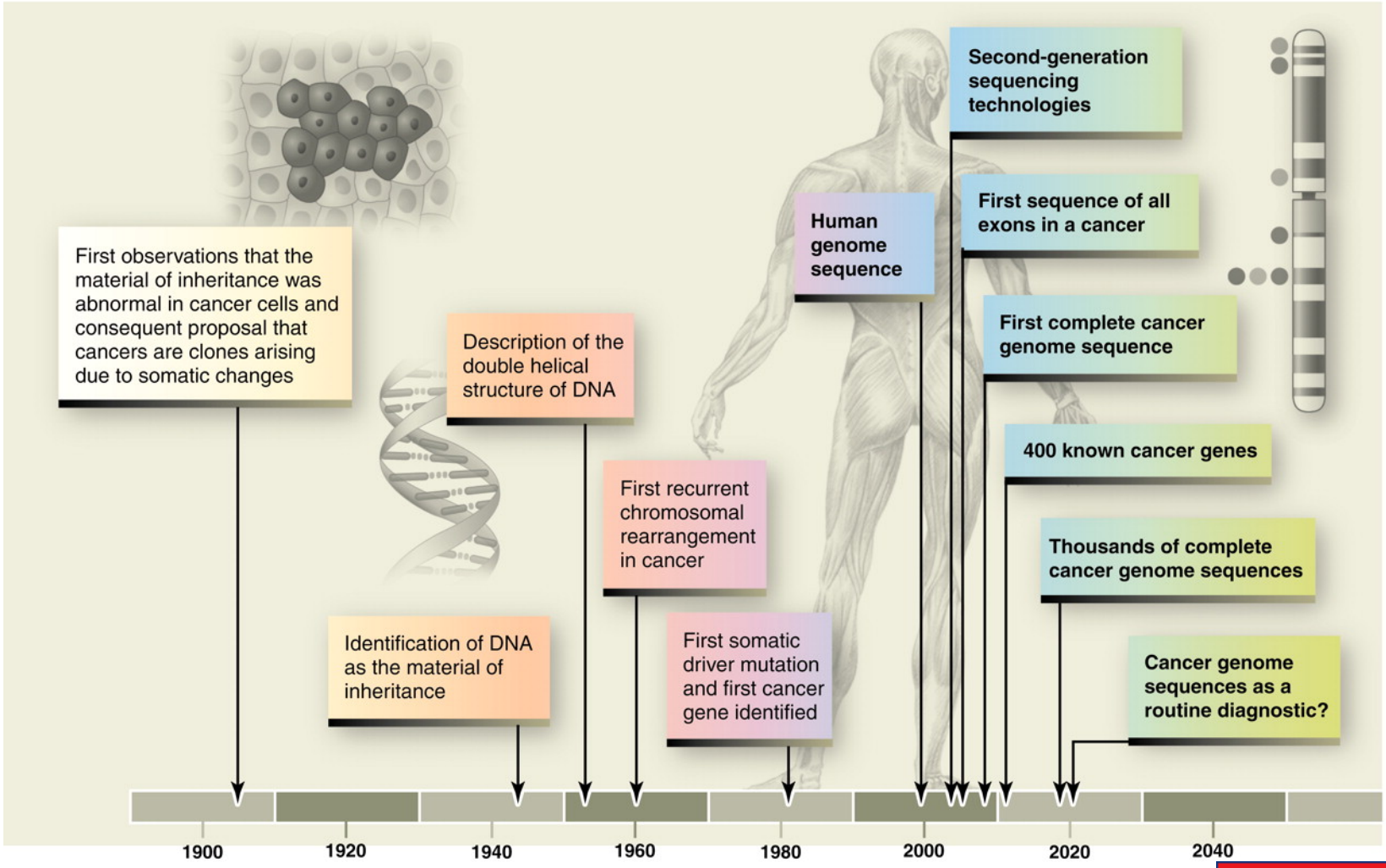
Figure 2. Fiber analysis results by gender and tumor site. The red bars and associated numbers indicate cases with an elevated asbestos fiber content, whereas the blue bars and associated numbers indicate cases with fiber content within the range of our reference population.

Published in: Alyssa Kraynie; Gustaaf de Ridder; Thomas Sporn; Elizabeth Pavlisko; Victor L. Roggli; *Ultrastructural Pathology* Ahead of Print DOI: 10.3109/01913123.2016.1154633

Bottom Line – More Genetic Data Will Inform Us All

- With all due respect, lawyers, courts and other experts completely failed to project the future as to mesotheliomas and the litigation
  - SEER data show mesotheliomas well beyond the projections of the "best and brightest"
  - bankruptcy trusts running out of money
- Era of genomics has arrived
  - Today, researchers can see things no one could see 5-10 years ago
  - costs to acquire genomic data have fallen dramatically
- More genomic data arriving now and over the next few years
  - TCGA analysis specifically regarding mesotheliomas (but only 30x coverage for sequencing)
  - new data will arrive from myriad genomic projects underway
  - new genomic projects will arise
  - lab on a chip technology will provide even more data

Fig. 1 Time line showing key events in the investigation of the cancer genome.



Michael R. Stratton Science 2011;331:1553-1558

Published by AAAS

Another Source for More Data: Human Exposome Project

The Human Exposome Project Is Aimed at Collecting Genetic and Epigenetic Data During and After Exposures

- IARC's Human Exposome Project has its roots in a 2005 article outlining improved methods to seek better answers as to sources of diseases
  - http://humanexposomeproject.com/

- "The human exposome is the environmental equivalent of the human genome. It is a representation of the complex exposures we are subjected to throughout our lives, including our diet, lifestyle factors, and social influences. It also incorporates how our bodies respond to these challenges."
- Methods now used are better experiments than old days - new method exposes the test creature AND measures impacts during and after the exposure
  - – blood, urine, proteins, DNA, RNA, microRNA, etc
- Work underway in the US at places such as Emory and Georgia Tech

- Consider the impacts of automated sensors – e.g. wearable devices
- Consider the impacts when implanted sensors yield even more data

Changing Nature of Evidence, and Examples of Statement in the Scientific Literature

Changing Nature of Evidence, and Low Dose Issues

- Nature of how we define "evidence" is changing
  - Both plaintiffs and defendants are relying on individual level genetic data,
  - Population and individual level experiments with gene "knock-in/out" animals
  - CRSPR – genetic editing and testing
  - Both plaintiffs and defendants relying on molecular/mechanistic evidence, across multiple toxins
  - "Lab on a chip" technology is fast, cheap and informative
  - Systems biology research across multiple toxins to find common mechanisms
  - FDA now make some approval decisions based on "real world" evidence without phase III trials

- Yes, there is evidence that genomic factors by themselves can "cause" mesothelioma

- Yes, there is evidence that low dose exposures will produce mesotheliomas in some persons with less robust genomes

- New, genomic epidemiology is needed

- More systems biology approaches to understanding causation pathways for a wide range of materials (asbestos, nano-materials, other fibers)

Carbone and Yang - 2015 - BAP1 and Low Dose Cases

The *Latest Developments* paper in 2015 by Carbone and Yang et al states the following:

> "Thus, since germline BAP1 mutations lead to an altered immune response following deposition of asbestos in tissues, interfering with this immune response might help prevent or delay MM in individuals carrying BAP1 mutations. **We found that BAP1+/- mice exposed to low doses of asbestos developed MM at a similar rate as wild type mice exposed to ten times higher doses [76]. Therefore, these findings support the hypothesis that germline BAP1 heterozygosity increases susceptibility to the carcinogenic effects of low dose [s] of asbestos.**"

Bononi, A., Napolitano, A., Pass, H. I., Yang, H., & Carbone, M. (2015). Latest developments in our understanding of the pathogenesis of mesothelioma and the design of targeted therapies. *Expert Review of Respiratory Medicine*, *9*(5), 633–654. http://doi.org/10.1586/17476348.2015.1081066

Peto, Carbone and Yang - 2016 - BAP1 and Low Dose Cases

The abstract of the 2016 *Consensus* paper by Peto, Carbone and Yang et al states the following:

- Abstract:
- ***
- "Genetics plays a critical role in MM when the disease occurs in carriers of germline BRCA1 associated protein 1 mutations.
- Moreover, it appears likely that, in addition to BRCA1 associated protein 1, other yet unknown genetic variants may also influence the individual risk for development of MM, especially after exposure to asbestos and related mineral fibers.

- Carbone M, Kanodia S, Chao A, et al. Consensus Report of the 2015 Weinman International Conference on Mesothelioma. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*. 2016;11(8):1246-1262. doi:10.1016/j.jtho.2016.04.028.

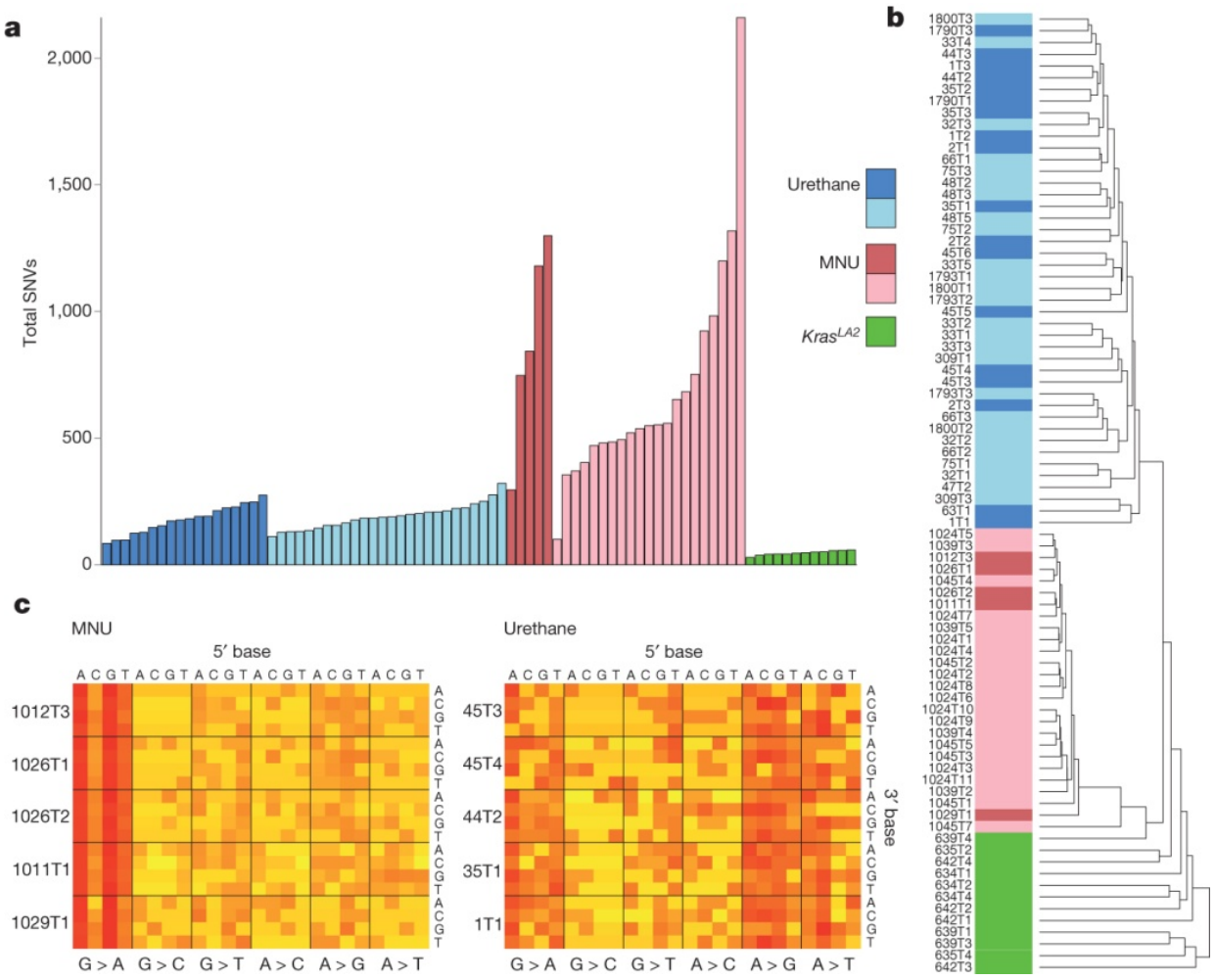
Carbone and Yang - 2017 - BAP1 and Low Dose Cases

The abstract of the 2017 *Recent Highlights* paper by Carbone and Yang et al states the following:

- Abstract: "Recent discoveries have elucidated some of the mechanisms responsible for the development of mesothelioma. These discoveries are: (I) the critical role of chronic inflammation in promoting mesothelioma growth, driven by the release of high mobility group box protein-1 (HMGB1) following asbestos deposition in tissues and its potential role as a biomarker to identify asbestos exposed individuals and mesothelioma patients;
- (II) the discovery that inherited heterozygous germline mutations of the deubiquitylase BRCA- associated protein 1 (BAP1) cause a high incidence of mesothelioma in some families; and that
- (III) germline BAP1 mutations lower the threshold of asbestos required to cause mesothelioma in mice, evidence of gene X environment interaction.
- These findings together with the identification of novel serum biomarkers, including HMGB1, Fibulin-3, etc., promise to revolutionize screening and treatment of this malignancy in the coming years."
- Carbone M, Yang H. Mesothelioma: recent highlights. Ann Transl Med 2017;5(11):238. doi: 10.21037/ atm.2017.04.29

Examples of the Arrival of "Molecular Signatures" In Diseases

Differences in mutation burden and spectra between carcinogen and genetic models.



PMK Westcott *et al. Nature* **000**, 1-4 (2014) doi:10.1038/nature13898

nature

Proof of Principle – Finding Signatures for Tumors Caused by a "Toxin"

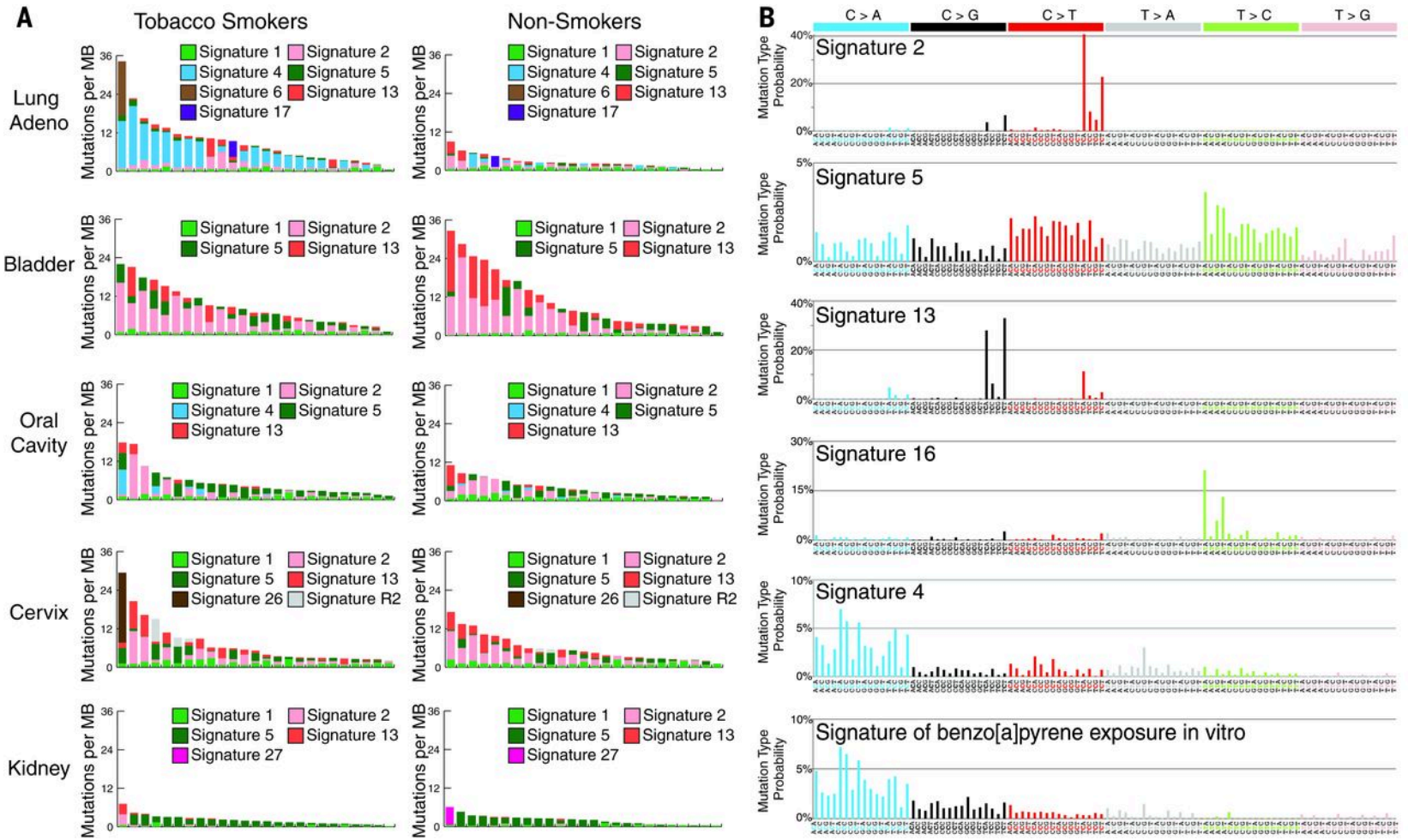
- As reported in a 2015 paper in Nature, researchers asked: can whole-exome sequencing and computers identify signature differences between tumors caused by two well-known "toxins" and tumors from an inherited (germline) mutation?
- Yes, is the proof of principle answer for KRAS mutations and these two toxins
- Westcott, The mutational landscapes of genetic and chemical models of Kras- driven lung cancer, Nature, 2015 Jan 22;517(7535):489-92. doi: 10.1038/ nature13898.
- The abstract states, pertinent part:

"Here we performed whole-exome sequencing on adenomas from three mouse models of non-small-cell lung cancer, which were induced either by exposure to carcinogens (methyl-nitrosourea (MNU) and urethane) or by genetic activation of *Kras* (*Kras*^{LA2}).

Although the MNU-induced tumours carried exactly the same initiating mutation in *Kras* as seen in the *Kras*^{LA2} model (G12D), MNU tumours had an average of 192 non-synonymous, somatic single-nucleotide variants, compared with only six in tumours from the *Kras*^{LA2} model.

By contrast, the *Kras*^{LA2} tumours exhibited a significantly higher level of aneuploidy and copy number alterations compared with the carcinogen-induced tumours, suggesting that carcinogen-induced and genetically engineered models lead to tumour development through different routes…. "

Fig. 2 Mutational signatures associated with tobacco smoking.



Ludmil B. Alexandrov et al. Science 2016;354:618-622

"Mutational signatures associated with tobacco smoking in human cancer"

- Alexandrov LB, Ju YS, Haase K, Van Loo P, Martincorena I, Nik-Zainal S, Totoki Y, Fujimoto A, Nakagawa H, Shibata T, Campbell PJ, Vineis P, Phillips DH, Stratton MR. Mutational signatures associated with tobacco smoking in human cancer. Science. 2016 Nov 4;354(6312):618-622.

- **"Abstract**
- Tobacco smoking increases the risk of at least 17 classes of human cancer.
- We analyzed somatic mutations and DNA methylation in 5243 cancers of types for which tobacco smoking confers an elevated risk.

- Smoking is associated with increased mutation burdens of multiple distinct mutational signatures, which contribute to different extents in different cancers.

- Smoking is associated with limited differences in methylation."

Seeking “Molecular Signatures” for Silica Induced Harm versus Asbestos Induced Harm

Search for Signature(s) for Silica Injuries versus Asbestos Injuries

- Researchers looking at diseased lungs for molecular markers related to disease caused by silica v. asbestos (Brooke Mossman lab)

- "Utilization of overall gene expression, unsupervised hierarchical cluster analysis and integrated pathway analysis revealed gene alterations that were common to both minerals or unique to either mineral."

- "Our findings reveal that both minerals had potent effects on genes governing cell adhesion/migration, inflammation, and cellular stress, key features of fibrosis. Asbestos exposure was most specifically associated with aberrant cell proliferation and carcinogenesis, whereas silica exposure was highly associated with additional inflammatory responses, as well as pattern recognition, and fibrogenesis."

- Indications for distinct pathogenic mechanisms of asbestos and silica through gene expression profiling of the response of lung epithelial cells, Hum. Mol. Genet., Mar 1;24(5):1374-89. doi: 10.1093/hmg/ddu551. Epub 2014

Liquid Biopsy:
Will Earlier Detection Change the Asbestos Litigation System?

Very Early Detection of Cancer - Liquid Biopsy Is Arriving

- "Liquid biopsy" process is intended to provide very early detection of cancers, with a goal of finding cancers long before symptoms are noticed by the person

- "Liquid biopsy" process uses new technology to cull through blood samples, seeking cancer cells "shed" by a tumor during its day to day processes

  - Cancer cells are found
  - Cancer cells are sequenced using whole genome sequencing
  - Artificial intelligence evaluates the results and learns continuously

- Liquid biopsy has drawn billions of dollars of investments from biotech industry leaders, including Illumina, Roche, Foundation Medicine, and many more

- One of the companies (Grail) has been headed up by a person who:
  - 1) lost his wife to cancer in her 40s,
  - 2) oversaw the projects that created Google Maps, Google Earth, and
  - 3) is a graduate of the University of Illinois computer science program, who
  - 4) challenges teams "to find a better way."

Grail Liquid Biopsy Data Released at AACR 2018

- Grail released some liquid biopsy data at AACR in Chicago in April 2018
- "GRAIL is a healthcare company whose mission is to detect cancer early, when it can be cured. GRAIL is using the power of high-intensity sequencing, population-scale clinical studies, and state-of-the-art computer science and data science to enhance the scientific understanding of cancer biology, and to develop and commercialize pioneering products for the early detection of cancer. For more information, please visit www.grail.com.
- https://www.businesswire.com/news/home/20180516006485/en/GRAIL-Present-New-Data-Circulating-Cell-free-Genome

Detection Rates (Sensitivity) for Cancers at Stages I-III at 98 Percent Specificity with Prototype Whole-Genome Bisulfite Sequencing Assay

Cancer Type (N)	Detection Rate (95% Confidence Interval)
Ovarian (10)	80% (44-98%)
Liver (5)	80% (28->99%)
Lymphoma (16)	69% (41-89%)
Multiple Myeloma (11)	64% (31-89%)
Pancreatic (10)	60% (26-88%)
Colorectal (27)	63% (42-81%)
Esophageal (19)	58% (34-80%)
Head and Neck (9)	56% (21-86%)
Breast (333)	21% (17-26%)
Triple-negative (48)	56% (41-71%)
HER2-positive (56)	34% (22-48%)
Hormone receptor-positive/ HER2-negative (217)	11% (7-16%)

More Grail Liquid Biopsy Data – ASCO - Chicago - June 2018

- At ASCO, Grail will release more data about its "Circulating Cell-free Genome Atlas"
- "The study has enrolled more than 10,000 people so far. (The goal is 15,000 by the end of this year.)
- With blood samples from 878 people with newly diagnosed cancer and 580 people without the disease, GRAIL performed three different kinds of tests that analyzed DNA across the entire genome.
  - One [test] looked for mutations in about 500 known cancer genes
  - Second [test] detected abnormal numbers of copies of genes
  - Third [test] analyzed patterns of methylation, which are chemical tags on DNA that turn genes off or on"
- https://www.sciencemag.org/news/2018/04/blood-test-shows-promise-spotting-early-cancers

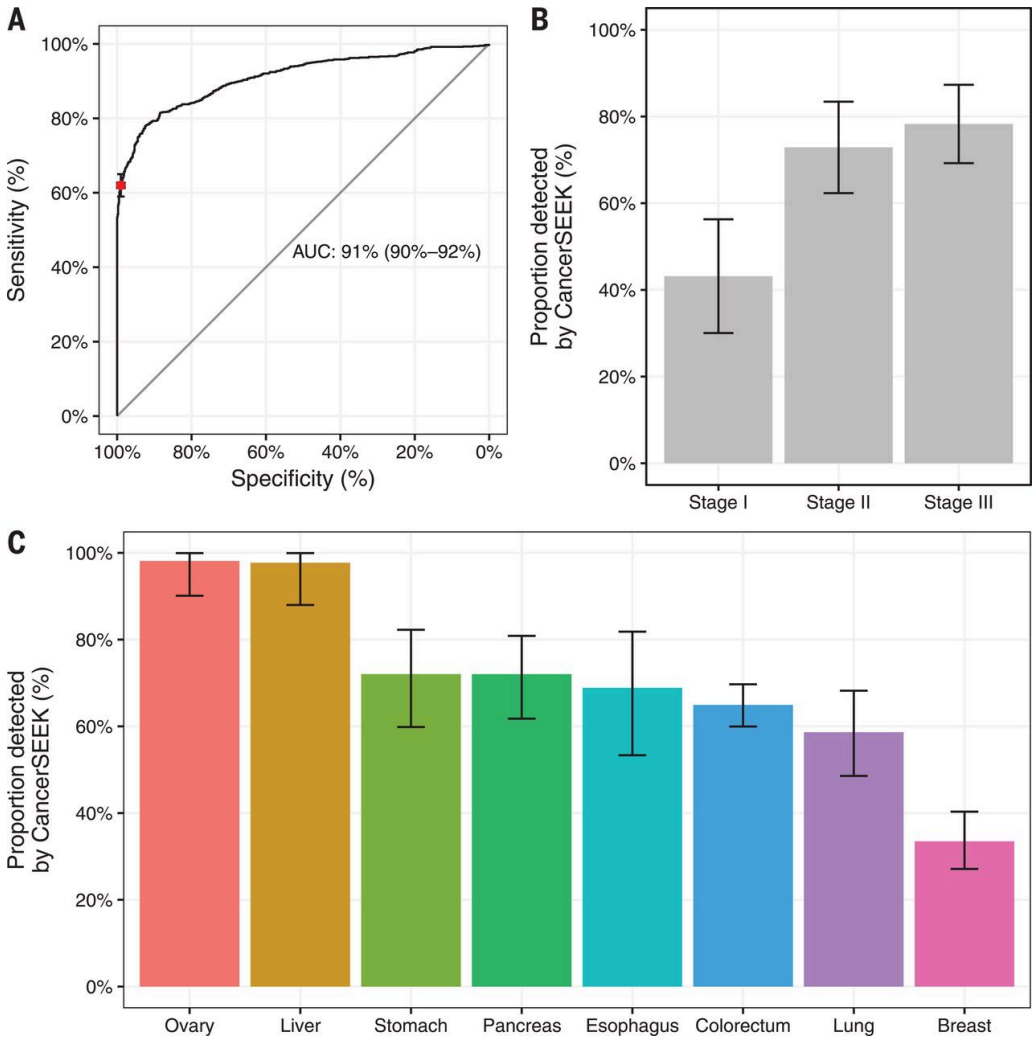
Detection and localization of surgically resectable cancers with a multi-analyte blood test

by Joshua D. Cohen, Lu Li, Yuxuan Wang, Christopher Thoburn, Bahman Afsari, Ludmila Danilova, Christopher Douville, Ammar A. Javed, Fay Wong, Austin Mattox, Ralph H. Hruban, Christopher L. Wolfgang, Michael G. Goggins, Marco Dal Molin, Tian-Li Wang, Richard Roden, Alison P. Klein, Janine Ptak, Lisa Dobbyn, Joy Schaefer, Natalie Silliman, Maria Popoli, Joshua T. Vogelstein, James D. Browne, Robert E. Schoen, Randall E. Brand, Jeanne Tie, Peter Gibbs, Hui-Li Wong, Aaron S. Mansfield, Jin Jen, Samir M. Hanash, Massimo Falconi, Peter J. Allen, Shibin Zhou, Chetan Bettegowda, Luis A. Diaz, Cristian Tomasetti, Kenneth W. Kinzler, Bert Vogelstein, Anne Marie Lennon, and Nickolas Papadopoulos

Science
Volume 359(6378):926-930
February 23, 2018

Published by AAAS

Fig. 2 Performance of CancerSEEK.



Published by AAAS

Joshua D. Cohen et al. Science 2018;359:926-930

Recap and Looking Ahead

Recap and Looking Ahead

- Watch individual trials and Bestwall chapter 11 issues regarding mesotheliomas
- Watch for Marchant and Hartley book on genetics in personal injury cases
- Expect to hear much more about BAP1
  - Testa lab - knockout mouse studies with 3 genetic variants inserted and chrysotile fibers injected into peritoneum
  - TCGA paper on mesotheliomas, including subset of women
  - More findings about germline mutations from myriad genome studies in progress
  - More papers from other leading researchers (Carbone, Roggli, MMSK)

- Testing only for BAP1 omits valuable data – broad analysis is better
  - Hundreds of genes are known factors in carcinogenesis
  - for mesothelioma, think about TP53, CDKNA2A, SNXX, VISTA, BRCA1 & 2, and others

- Watch for more on the role of smoking and mesothelioma in some persons
- Watch for more focus on epigenetics as a driver of mesotheliomas
- Watch for the arrival of liquid biopsy and screening programs (perhaps with a focus on ovarian cancers)
- Watch for more on somatic signature mutation patterns for:
  - lung cancers and asbestos
  - tobacco

Data Generated in Litigation Should Be Used

- Following statements are purely my personal opinion formed after multiple encounters with cancer in my personal life, as well as professional knowledge

- Genomic and exposure data generated in litigation should be used to advance science

- Courts should approve genetic testing when shown a case with facts that suggest genetic factors strongly involved
  - Young (under 50) and/or very low exposure
- Privacy concerns are largely misplaced
    - Any excellent cancer center already looking at genes to treat cancers effectively
      - (that is the science behind precision medicine)
      - Foundation Medicine multi-gene test now FDA approved
    - most people with cancer want their experience to help prevent other cancers
- Genetic data is objective — litigation does not change the process or the outcome
  - for sequencing DNA
  - for analysis of RNA
  - for analysis of epigenetic factors, such as microRNA

- Unlike doctors and genetic researchers, law firms and others actually are good investigators
  - Testimony and interrogatory answers can shed real light on whether a person actually was or was not exposed to asbestos fibers
  - Tissue samples should be used and stored for future use

Invest in Mesothelioma Research and Treatment

- Mesotheliomas are not going away

- Most federal and state budgets shrinking

- Carbone et al: “Additionally, although much needed novel therapeutic approaches for MM are being developed and explored in clinical trials, there is a critical need to invest in prevention research, in which there is a great opportunity to reduce the incidence and mortality from MM.

- Mesothelioma Applied Research Foundation
  - not interested in litigation
  - grants to leading researchers

Background and Disclosures

- Since 1984, trial lawyer for commercial litigation and mass tort cases for very large corporations involved in manufacturing (no work for insurers)
  - many business cases; several related to toxic tort indemnification or insurance
  - numerous cases taken to final judgment, including jury verdicts and non-jury final judgements rendered in federal and state cases, arbitration cases and asbestos-related chapter 11 cases

- Practicing lawyer at my law firm - LSP Group LLC
- Partner in ToxicoGenomica - expert services for issues involving genomics in civil law
- Senior advisor in national consulting firm (Nathan Inc.) – wide range of experts

- Pro bono director of Triage Cancer, a not for profit focused on cancer issues for persons seeking to survive cancer
  - www.TriageCancer.org
- Pro bono legal work and lawsuits for persons with cancer and/or diabetes against public and private health insurers that deny access to care
- Numerous science/law/cancer presentations for groups such as Biden Blue Ribbon panel on cancer, academic workshops and cancer advocacy groups

GlobalTort Blog

- GlobalTort blog is located at www.GlobalTort.com
- Blog focuses on intersections between science, law, and other disciplines
- Numerous articles on asbestos
- Updated 3-5 times per week, most weeks

Questions or follow-up?

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