Genomics for Causation Analyses in Asbestos Cases

Perrin Mid-Atlantic Asbestos Conference

July 17, 2019

Len van Zyl, Ph.D.

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Genomic Analyses ➡ Precision Medicine

Transition from “One-Size-Fits-All” to Individualized Medical Treatment Known as “Precision Medicine”

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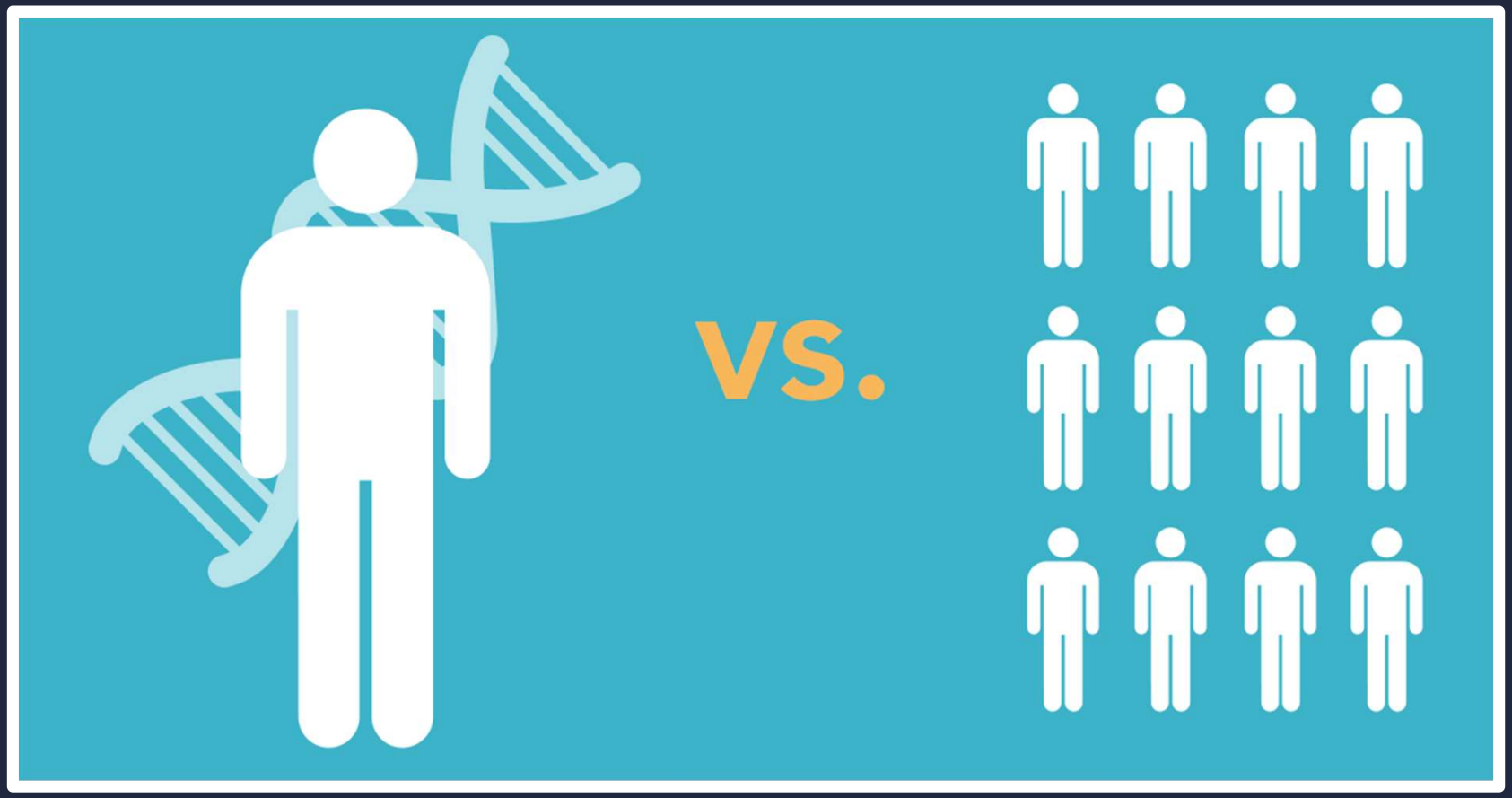

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WHY PRECISION MEDICINE?

BECAUSE:

Each and Every Person is Genetically Unique

Vast Increases in Genomic Sequencing Mean Vast Increases in Genetic Data and Genes Involved in Cancers/Diseases

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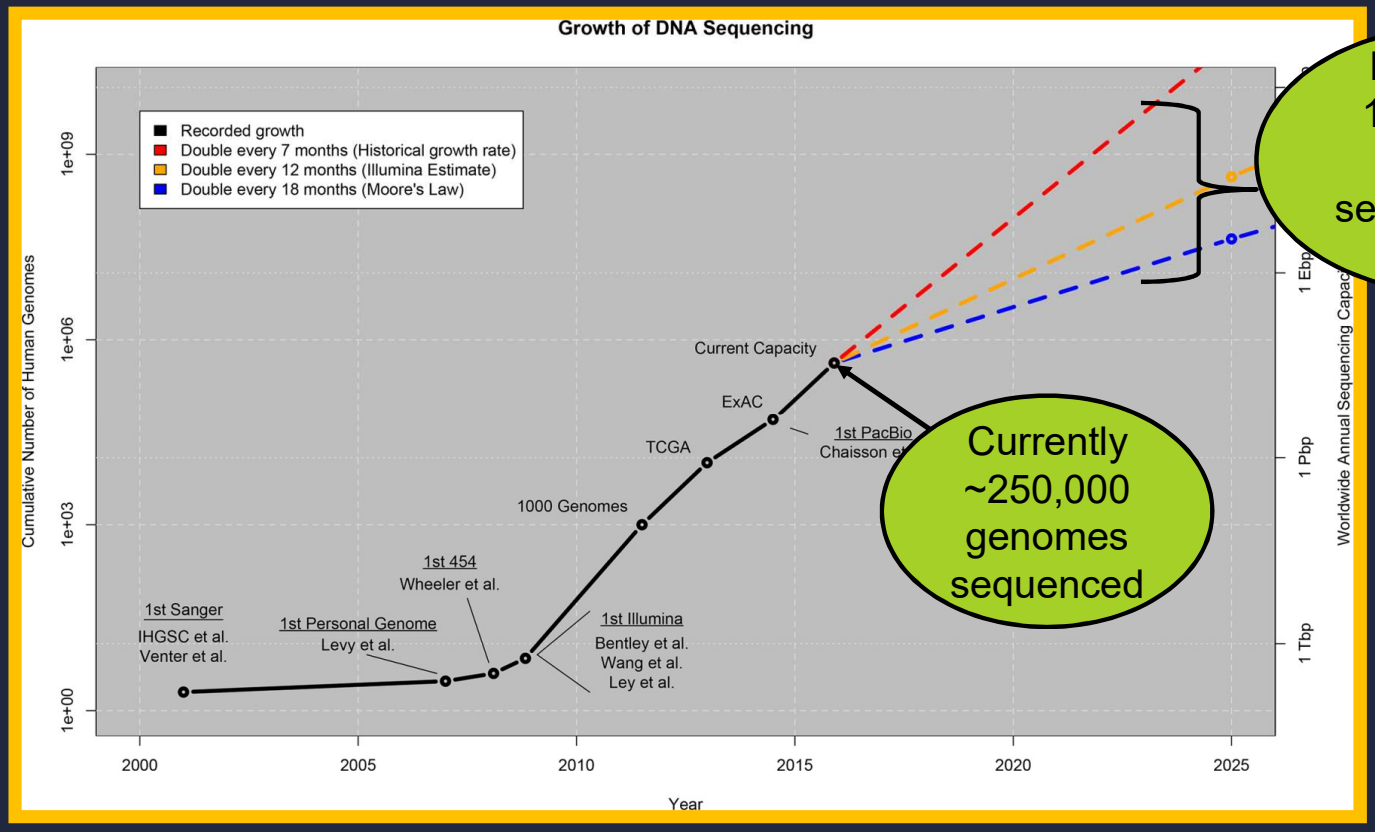

Genomic Data

Raw Whole Genome	~3B base pairs ~30x + coverage ~100 gigabytes
Whole Genome	~3B base pairs ~700 megabytes
Whole Exome	~30M base pairs ~7 gigabytes
Variant Call File	~3M Variants ~125 megabytes

Stephens *et al.* 2015

For Litigation, Genomic Analysis Can Add…

- Signatures for likely toxicant exposure
- Alternative causation (genetic causes of cancers)
- Objective data to show susceptibility or protective genes relevant to particular toxicants

“Make Visible the Invisible!”

Courts and Commentators Mainly Approve Genetic Testing

Increasing National Recognition For The Importance of Genomic Analyses in Tort Cases

- **"The growing knowledge of genetic factors and their interaction with environmental factors has the power to forever change mass tort and class action litigation."**
  - Dr. Bob Wassman – MD, medical geneticist and founder, CEO or CMO for 6 businesses
  - https://news.bloomberglaw.com/class-action/insight-genomics-could-make-toxic-mass-tort-class-actions-harder-to-win (March 20, 2019)

- ***"Incomplete Penetrance: Whole-Exome Sequencing and Federal Courts"***,
  - Andrew Gendron & Thomas M. Morgan (January 2019)
  - https://www.scribd.com/document/415649355/Gendron-and-Morgan-Whole-Exome-Sequencing-and-Federal-Courts-FTD-January-2019

- ***"The Use of Genetic Evidence to Defend Against Toxic Tort Claims—Parts I-III",*** **Intell. Prop. & Tech. L.J. (2017). 8**
  - Susan E. Brice & Dr. Whitney V. Christian
  - Available at: https://www.bryancave.com/images/content/9/9/v2/99117/IP-Reprint-Article-complete.pdf

- **"Genetic Data in Toxic Tort Litigation", THE BRIEF (ABA), Winter 2016, 22-31**
  - Gary E. Marchant
  - https://www.scribd.com/document/415652109/Marchant-Genetics-in-Toxic-Tort-Litigation-2016

Multiple Court Orders Support Genomic Testing, and Some Plaintiff Firms Seek Pre-Litigation Genomic Testing

- Most state and federal court systems have statutes or court rules that require plaintiff to undergo independent medical review procedures, which may include genomic analyses
- Many courts have entered orders approving genomic testing in personal injury cases, but some disapprove based on case specific facts
  - benzene cases, asbestos cases, birth defect cases, prescription drug cases , vaccine cases
    - *e.g*. Thrash v. Boeing Co, 2018 WL 2573097 (approved *BAP1* testing)

- **Some "sophisticated" plaintiff firms use genetic testing in anticipation of litigation**, as illustrated by two recent cases involving a form of genomic testing (CMA) obtained by plaintiffs in birth defect cases. See Andrew Gendron & Thomas M. Morgan, *Whole-Exome Sequencing and Federal Courts*, For the Defense (January 2019) at 22

Privacy Arguments Do Not Defeat Genetic Testing

- We are not aware of any court order that disapproved genetic testing based on a privacy argument by a plaintiff
  - please let us know if you find one
- In fact, some courts have considered privacy issues at length and then approved genetic testing
  - see *e.g.* ruling in *Ortwein* mesothelioma case handled by Kazan firm for Ortwein family
  - https://www.scribd.com/doc/269179075/Ortwein-Order-of-12-22-2014-Granting-Motion-to-Compel-Production-of-Lung-Tissue-Sample-re-BAP1
- The Ortwein family ultimately chose to go to trial and proactively disclosed familial genetic mutations as part of arguing "susceptibility" to low doses of asbestos

Genomic Patterns & Asbestos Related Cancers

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Epidemiologists Acknowledge Genomic Variables Influence Cancers in Individuals

- Suresh Moolgavkar – MD, PhD - well published and respected MD & cancer epidemiologist at Fred Hutchinson, frequent expert witness, senior fellow & principal scientist at Exponent, stated:

"large differences in susceptibility are determined by major gene defects or by events occurring in embryonic life that alter populations of critical cells…."

International Journal of Epidemiology 1425-26 (April 2015)

Genomic Testing Can Reveal Causation Related Mutations & Patterns in Multiple Types of Cancers

- Mesothelioma
  - Numerous germline mutations are now known to be involved (some focus on 30 or so, we see more)
  - More genes will be identified and more subtypes and patterns are emerging
  - But some individuals have unique sets of germline and somatic mutations
  - We believe more data will arrive this year on patterns in and differences between mesotheliomas
- Ovarian
  - Generally speaking, ovarian cancers are considered the most genetically driven cancers
  - Multiple subtypes of ovarian cancer with varying characteristics
- Lung
  - Some germline mutations are well known to cause lung cancers (*e.g. KRAS*)
  - Some researchers have developed somatic signature mutation patterns for asbestos-induced lung cancers

Some Basics of Genetic Testing

Case Selection Criteria for Likely Genetic Predisposition

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Plaintiffs' Personal Medical history

- Early age at diagnosis (<50 yrs)
- Multiple primary tumors
- Bilateral/multiple rare cancers
- Constellation of tumors consistent with known cancer syndrome (e.g. hematologic cancers, breast and ovarian, mesothelioma, renal cell and melanoma, Lynch Syndrome spectrum of cancers)

Family Cancer History

- 2+ relatives on same side of family diagnosed with cancers
- Ancestry (*e.g.* Ashkenazi ancestry)

Genetic Testing – Simplified List

	Yes	No
Young age at cancer onset?		
Evidence of exposure?		
Family medical history of related diseases?		
Previous genetic diagnostics?		
Tissue sample availability? Tissue type?		
Lifestyle/behavioral risks?		

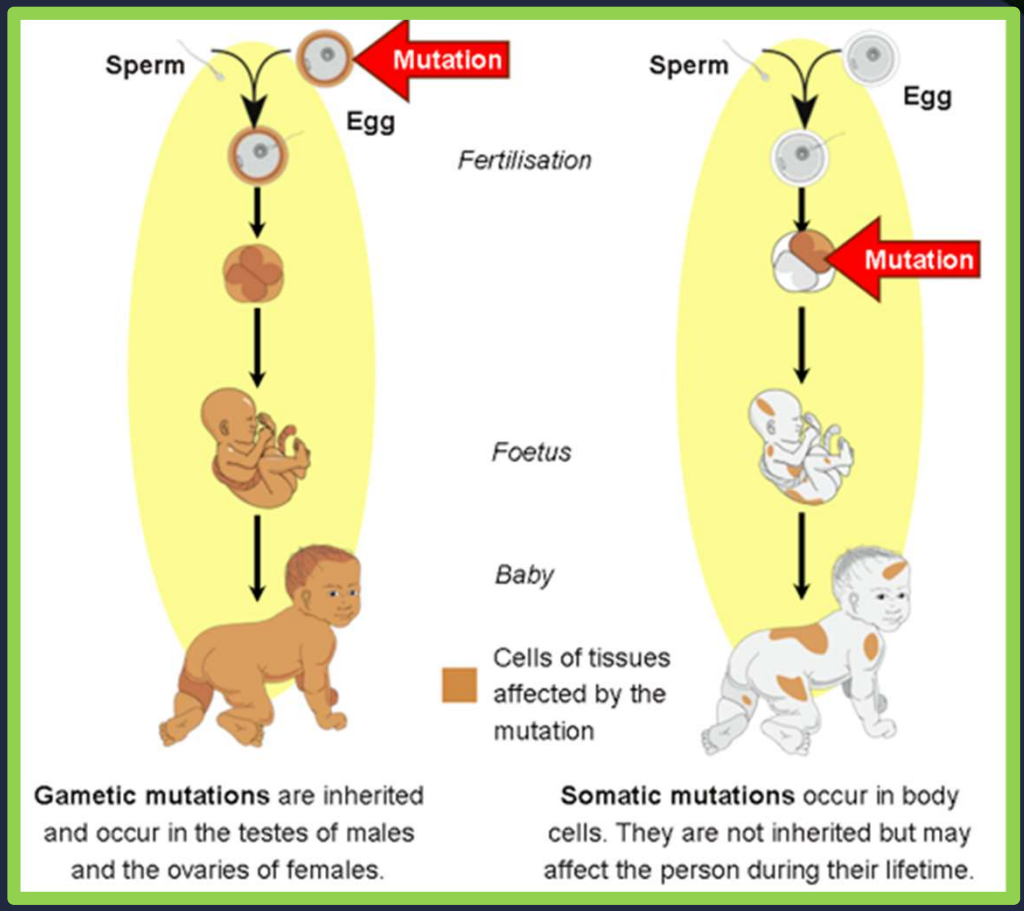
Two Relevant Types of Gene Mutations

Germline Mutations

- Present in egg or sperm
- Are heritable
- Cause hereditary cancer syndromes

Somatic Mutations

- Occur in non-germline tissues
- Are non-heritable
- Later onset



Predisposition vs. Susceptibility

Genetic Predisposition

- A person may inherit gene variants (mutations) that increase likelihood of developing a disease
- No toxicant required
- Not every carrier of a predisposing genetic variant(s) will get the disease (depends on the **penetrance** of the pathogenic variant)
- **PENETRANCE** is the proportion of individuals carrying a particular variant(s) (genotype) that express its associated trait/phenotype

Genetic Resistance/Susceptibility

- Genetic susceptibility determines how a person will "react to/metabolize" a particular toxicant (*i.e.* benzene metabolism)
- Some people will be susceptible, others resistant to a toxicant (protective alleles)

What's Needed for Germline Genetic Testing?

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- Non-cancerous DNA is needed in order to sequence **GERMLINE MUTATIONS** to determine genetic variants that a person received at conception
  - DNA is most easily extracted and sequenced from small amounts of blood (5ml)
  - DNA can be extracted from cheek cells (known as buccal swabs) and saliva but more effort is involved
  - non-cancerous DNA can readily be extracted from FFPET samples
  - Some times, but not always, DNA can be extracted from a cadaver (variables include how the embalming was done)
- **Multiple gene testing is the only logical and cost effective path; single gene testing is obsolete**

What's Needed for Somatic Mutation Testing?

- Cancerous tumor tissue is needed to sequence tumor tissue to **find SOMATIC MUTATIONS** in the cancer cells
  - Tumor tissue can be fresh or tissue from **Formalin Fixed Paraffin Embedded Tissue** (FFPET) blocks
  - Sometimes, tumor tissue also can be extracted from a cadaver (variables include how the embalming was done)
  - At any excellent cancer center, somatic mutation testing is usually performed as part of "**Precision Medicine**" decisions about how to treat that particular cancer in that particular person

Genetic Testing by Commercial Labs – Costs & Benefits

- **For litigation, there is ZERO value to test run for purposes of determining ancestry**
- For litigation, tests currently run by 23andme are almost always of no value, but could open doors
- Costs vary for commercial lab testing of germline "cancer genes"
  - different commercial labs test different combinations of genes for various purposes
  - commercial lab testing is rigid in terms of genes tested
  - commercial labs fail to test some genes that are key for specific cancers (*i.e.* mesothelioma)
  - commercial lab reports are rigid on timing to report, usually at least 6-8 weeks
  - commercial lab reports typically identify mutations found, but provide no analysis of relevance of mutations to causation
- **In some instances, results from a commercial test may provide useful insights into the appropriate scope of further genetic testing**

Genetic Testing By ToxicoGenomica/ArrayXpress Costs and Benefits

- ToxicoGenomica has performed genomic analysis and/or consulting in over 25 toxic tort cases
- **We strongly urge multi-gene testing (*i.e.* full genome or exome sequencing); sequencing results in all our cases have produced unexpected and compelling findings regarding multiple genes**
- Costs vary depending on various factors, including quality and source of DNA, time constraints, scope of genes tested (targeted gene panel, full exome or whole genome sequencing; RNA and miRNA gene expression etc.) and scope of report(s)

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Germline Genetic Testing

Sequencing Genes To Reveal Inherited Mutations That Can Cause Cancers

Inherited Cancer Predisposition Syndromes; Multiple Genes

- Inherited disease syndromes have been observed for hundreds of years
  - Today we know some arise from inherited mutations in 1 gene
    - e.g. cystic fibrosis, sickle cell, “bubble boy” immune deficiency
- Similarly, Inherited Cancer Predisposition Syndromes were observed for decades, but the involved genes were not yet known (*e.g.* familial breast cancers)
- **Today, we know many inherited cancer syndromes involve multiple genes**
- Today, large collections of genomic data, and new high speed genomic investigation tools, are revealing more patterns within cancers and thus revealing more genes and signature patterns involved in cancer syndromes and particular cancers

Broad Patterns in Hereditary Cancer Syndrome(s)

- Early age at cancer diagnosis (<50 yrs)
- Multiple primary tumors in that person
- Cancer in 2 or more relatives (on same side of family)
- Cancers are part of “constellation” of tumors consistent with specific cancer syndrome (e.g. melanoma with pancreatic cancers, breast and ovarian, colon with endometrial)
- Bilateral or multiple rare cancers
- Ancestry (e.g. Ashkenazi ancestry)

Mesothelioma:
Big Picture Genomic Data

Why is There Such Great Variability in Who Develops Mesothelioma?

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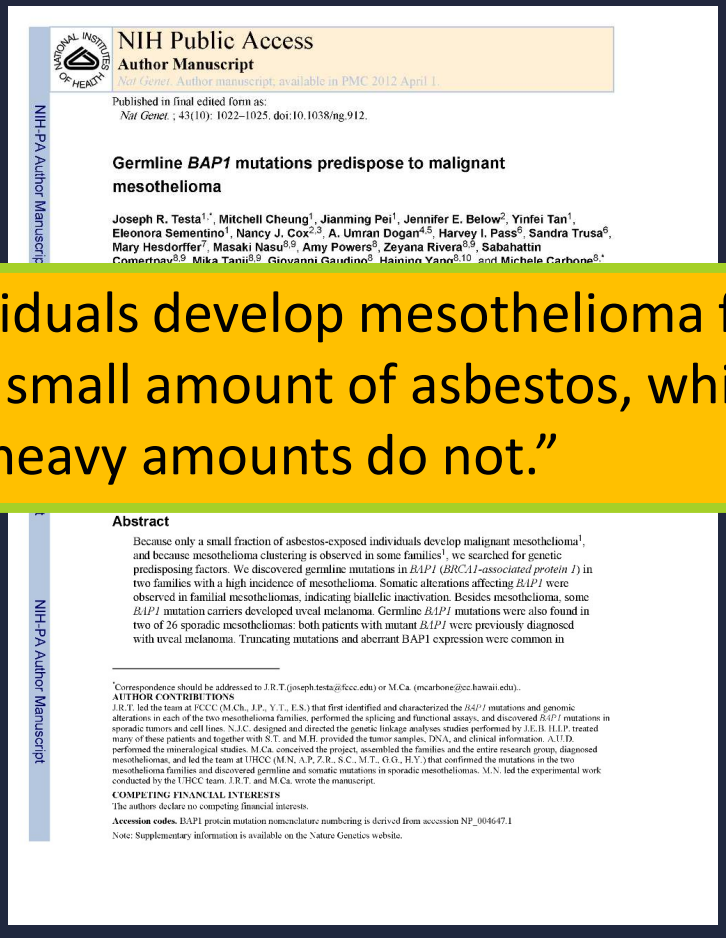

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Germline *BAP1* mutations predispose to malignant mesothelioma

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[8], Haining Yang[8,10], and Michele Carbone[8,*]

"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.

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Hereditary Predisposition to Mesothelioma

- *BAP1* was the first gene known to predispose to mesothelioma
- **Current data suggests that 5-20% of all mesotheliomas are initiated by germline mutations in genes known to cause onset of clinically, well defined Hereditary Cancer Predisposition Syndromes, in the absence of asbestos exposure** (Kraynie *et al*., 2016)
- Multiple published research studies have identified >60 genes known to initiate mesothelioma in a hereditary context (and more to come!)

Causation Issues

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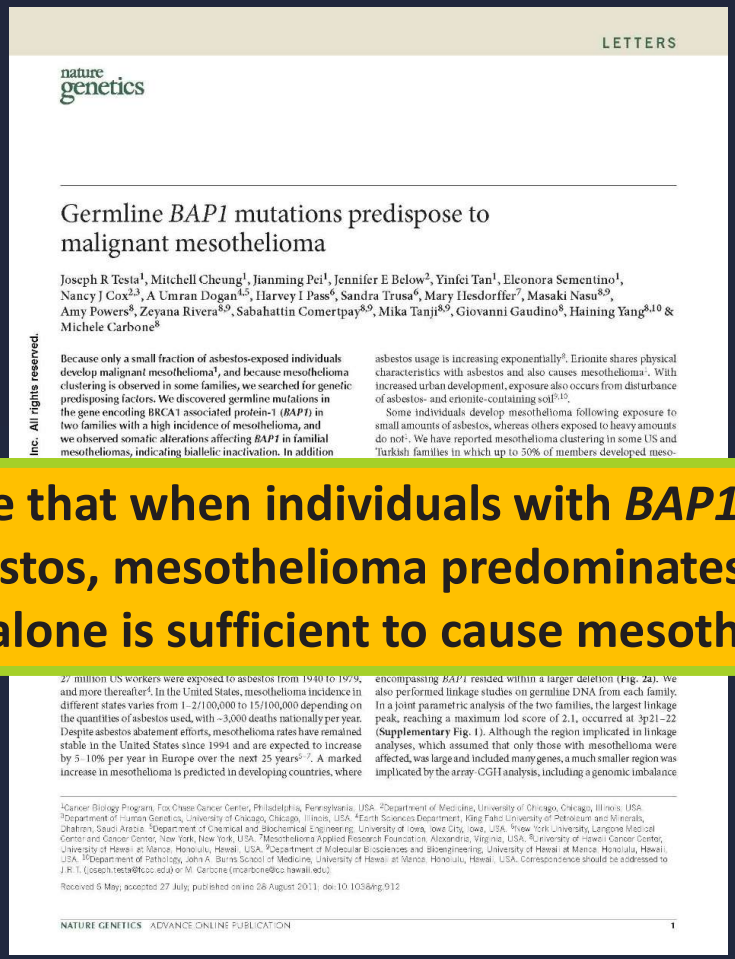

LETTERS

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 develop malignant mesothelioma[1], and because mesothelioma clustering is observed in some families, we searched for genetic predisposing factors. We discovered germline mutations in the gene encoding BRCA1 associated protein-1 (*BAP1*) in two families with a high incidence of mesothelioma, and we observed somatic alterations affecting *BAP1* in familial mesotheliomas, indicating biallelic inactivation. In addition

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

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[5–7]. A marked increase in mesothelioma is predicted in developing countries, where asbestos usage is increasing exponentially[8]. Erionite shares physical characteristics with asbestos and also causes mesothelioma[9]. With increased urban development, exposure also occurs from disturbance of asbestos- and erionite-containing soil[9,10].

Some individuals develop mesothelioma following exposure to small amounts of asbestos, whereas others exposed to heavy amounts do not[4]. We have reported mesothelioma clustering in some US and Turkish families in which up to 50% of members developed meso-

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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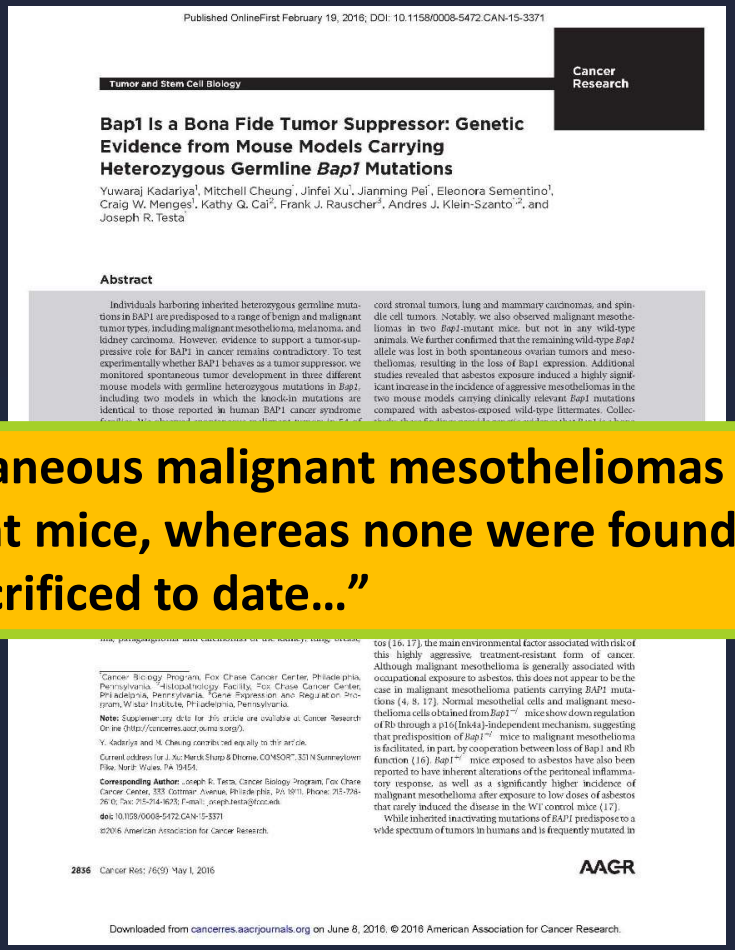
BAP1 Mutations Lead to Spontaneous Mesothelioma Tumors in Mice



Published OnlineFirst February 19, 2016; DOI: 10.1158/0008-5472.CAN-15-3371

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[2], Jinfei Xu[3], Jianming Pei[4], Eleonora Sementino[1], Craig W. Menges[1], Kathy Q. Cai[5], Frank J. Rauscher[6], Andres J. Klein-Szanto[5,7], and Joseph R. Testa[1]

Abstract

Individuals harboring inherited heterozygous germline mutations in BAP1 are predisposed to a range of benign and malignant tumor types, including malignant mesothelioma, melanoma, and kidney carcinoma. However, evidence to support a tumor-suppressive role for BAP1 in cancer remains contradictory. To test experimentally whether BAP1 behaves as a tumor suppressor, we monitored spontaneous tumor development in three different mouse models with germline heterozygous mutations in *Bap1*, including two models in which the knock-in mutations are identical to those reported in human BAP1 cancer syndrome

cord stromal tumors, lung and mammary carcinomas, and spindle cell tumors. Notably, we also observed malignant mesotheliomas in two *Bap1*-mutant mice, but not in any wild-type animals. We further confirmed that the remaining wild-type *Bap1* allele was lost in both spontaneous ovarian tumors and mesotheliomas, resulting in the loss of Bap1 expression. Additional studies revealed that asbestos exposure induced a highly significant increase in the incidence of aggressive mesotheliomas in the two mouse models carrying clinically relevant *Bap1* mutations compared with asbestos-exposed wild-type littermates. Collec-

"Two spontaneous malignant mesotheliomas were identified in *Bap1*-mutant mice, whereas none were found in any of the 43 WT mice sacrificed to date…"

Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, Pennsylvania. [2]Histopathology Facility, Fox Chase Cancer Center, Philadelphia, Pennsylvania. [3]Gene Expression and Regulation Program, Wistar Institute, Philadelphia, Pennsylvania.

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

An Example of Studies Plaintiffs Like and Defendants Will Need to Confront

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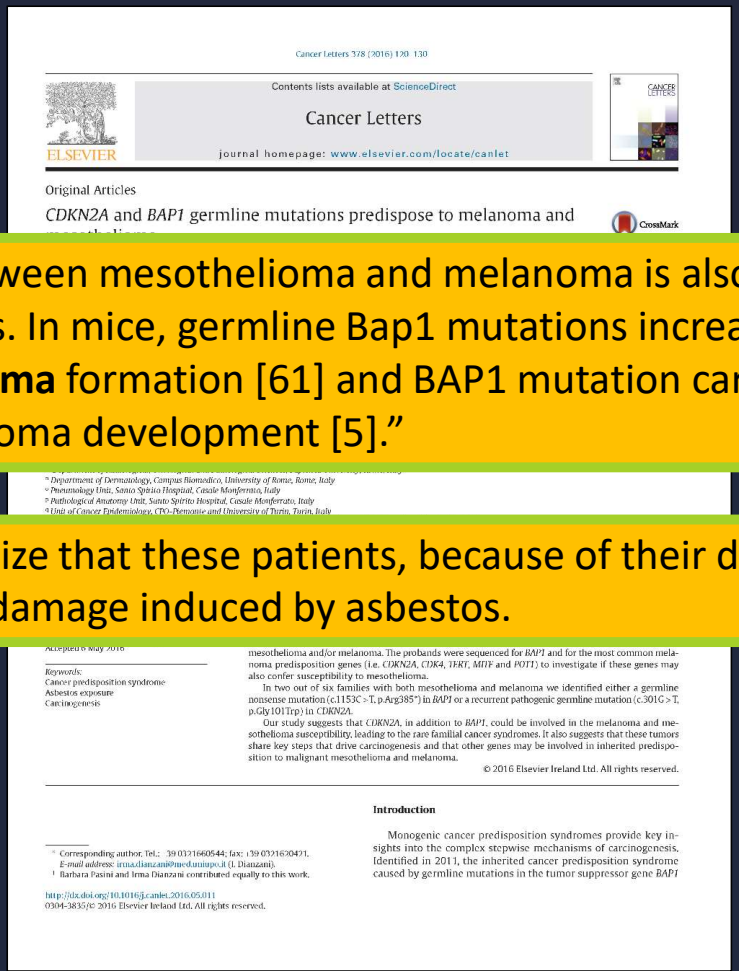

Cancer Letters 378 (2016) 129–130

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

CDKN2A and *BAP1* germline mutations predispose to melanoma and

"A common denominator between mesothelioma and melanoma is also the well-defined role of **environmental** carcinogens. In mice, germline Bap1 mutations increase the **susceptibility to asbestos induced mesothelioma** formation [61] and BAP1 mutation carriers may be prone to UV carcinogenesis and melanoma development [5]."

It is thus possible to hypothesize that these patients, because of their defect in DNA repair, were less able to repair DNA damage induced by asbestos.

Betti *et al*., 2017.
http://dx.doi.org/10.1016/j.canlet.2017.06.028

Betti *et al*., 2016

Examples of Plaintiff and Defense Arguments Regarding Cancers

Highly Contested BAP1 Issues - Holly Ortwein Case – 2016

- Mrs. Ortwein was 4th in her family to develop mesothelioma
- Possible low dose exposures included some intake at home and other possibilities 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 and some reframing
  - Good questions were posed by jurors - see Schwartz and Hartley article https://www.law360.com/articles/893614/jurors-in-toxic-tort-litigation-take-genetics-seriously
  - Case settled before testimony by defense expert (Dr. Feingold)

Example of Inherited Gene Argument by a Defendant

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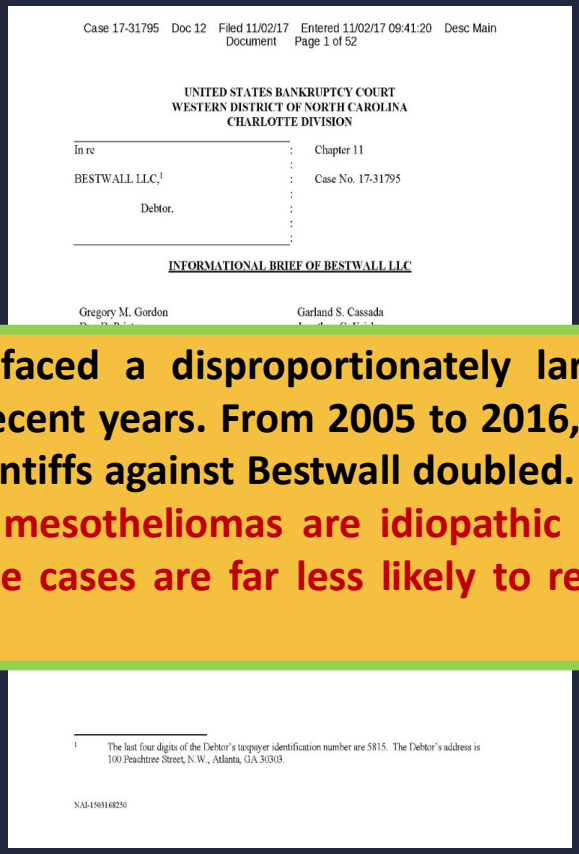

Case 17-31795 Doc 12 Filed 11/02/17 Entered 11/02/17 09:41:20 Desc Main
Document Page 1 of 52

UNITED STATES BANKRUPTCY COURT
WESTERN DISTRICT OF NORTH CAROLINA
CHARLOTTE DIVISION

In re	:	Chapter 11
BESTWALL LLC,[1]	:	Case No. 17-31795
Debtor.	:	

INFORMATIONAL BRIEF OF BESTWALL LLC

Gregory M. Gordon — Garland S. Cassada

[1] The last four digits of the Debtor's taxpayer identification number are 5815. The Debtor's address is 100 Peachtree Street, N.W., Atlanta, GA 30303.

NAI-1503168250

"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."

"Informational Brief" filed by Bestwall/Georgia Pacific on 11/2/17 (Footnote 35)
https://www.scribd.com/document/379701100/Bestwall-Georgia-Pacific-chapter-11-Doc-12c-Informational-Brief-as-Filed

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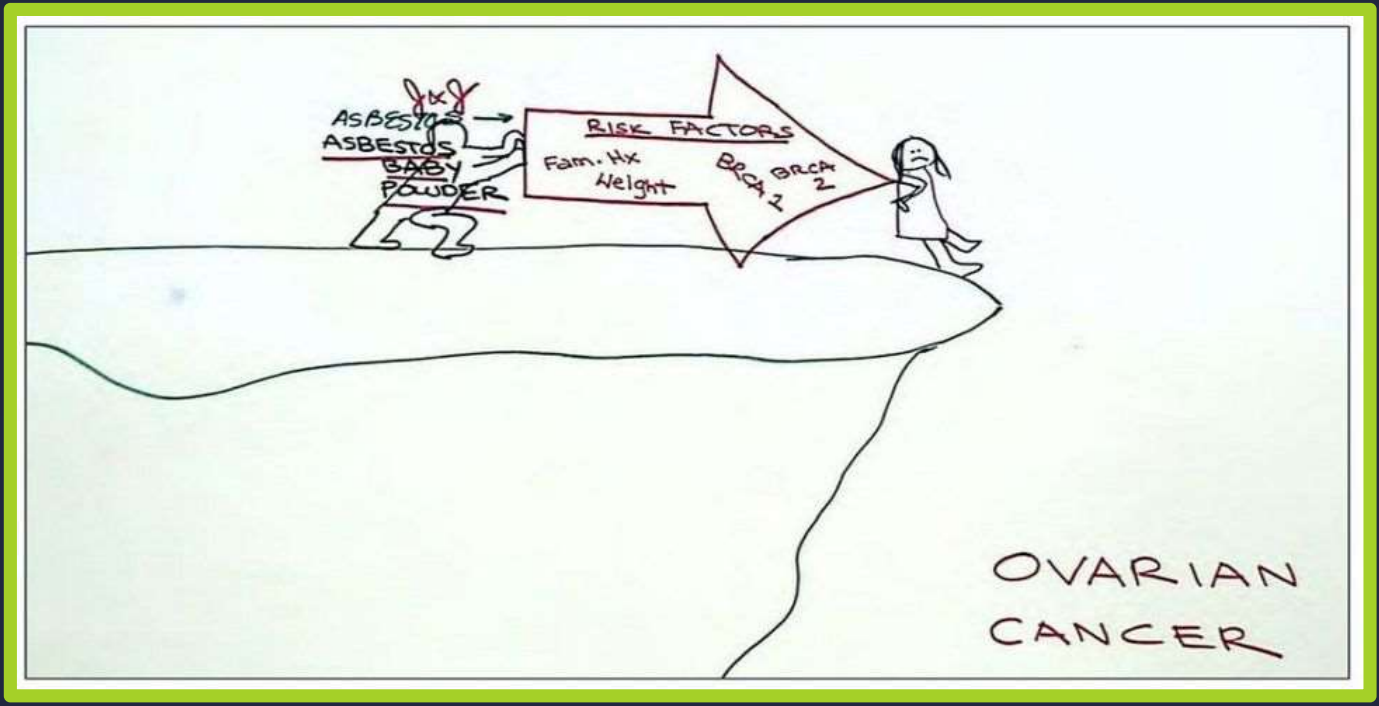
Example of Inherited Gene Argument by A Plaintiff



Mark Lanier

- Plaintiff argued “susceptibility”
- Note references to mutations of *BRCA1* and *BRCA2*
- Graphic used in trial of Ingham - 22 ovarian cancer cases

Multiple Genes Involved in Mesothelioma

Hereditary Predisposition to Mesothelioma Involves Multiple Genes

- Numerous germline variants (mutations) predispose to malignant mesothelioma: *BAP1*, *PALB2*, *BRCA1*, *FANCI*, *ATM*, *SLX4*, *BRCA2*, *FANCC*, *FANCF*, *PMS1*, *XPC* (Betti *et al*., 2017; Panou *et al*., 2018)
- *CDKN2A* predispose to both melanoma and mesothelioma *(*in addition to *BAP1* germline mutations) [Betti *et al*., 2016]
- Other yet to be discovered genetic factors no doubt exist

Hereditary Predisposition to Mesothelioma

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In 2018, Many Published Articles Demonstrated Hereditary Predisposition

- Inherited germline genetic mutations have a significant role in the development of mesothelioma (Kharazmi *et al*., 2018)
- 198 patients with mesothelioma showed mutations in 13 known cancer predisposition genes, including *BAP1 (*younger, minimal asbestos exposure, a second cancer, and peritoneal malignant mesothelioma (Panou *et al*., 2018)
- 3/88 patients with peritoneal mesothelioma had *ALK* gene fusions (rearrangements) – ALL lacked asbestos exposure
- Numerous mutations identified in **11 (non-BAP1)** "Cancer Census Genes" in a Belgian family with history of mesothelioma
- Infant with mesothelioma belonged to a family with known pathogenic mutations in *ATM* (gene known to increase risk of various malignancies
- Germline *PTEN* mutation in a patient with mesothelioma

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Identification of *ALK* Rearrangements in Malignant Peritoneal Mesothelioma

- There is a question as to the malignant peritoneal mesothelioma association with anaplastic lymphoma kinase (*ALK*) rearrangements
- Hung *et al*. (2018) identified in a large series of 88 consecutive patients with peritoneal mesothelioma, ***ALK* rearrangements to occur in 3% of cases that**:
  - **present in young women (25% of women younger than 40 years)**,
  - **lack asbestos fibers**,
  - **have no history of therapeutic radiation**,
  - **lack the typical cytogenetic and molecular abnormalities usually present in peritoneal mesothelioma (*e.g.* loss of chromosomal region 9p or 22q or genetic alterations in *BAP1*, *SETD2*, or *NF2*)**
- The Authors concluded that ALK rearrangements reveals a novel pathogenetic mechanism of malignant peritoneal mesothelioma with promise for targeted therapy

EWSR1 / FUS Rearrangements

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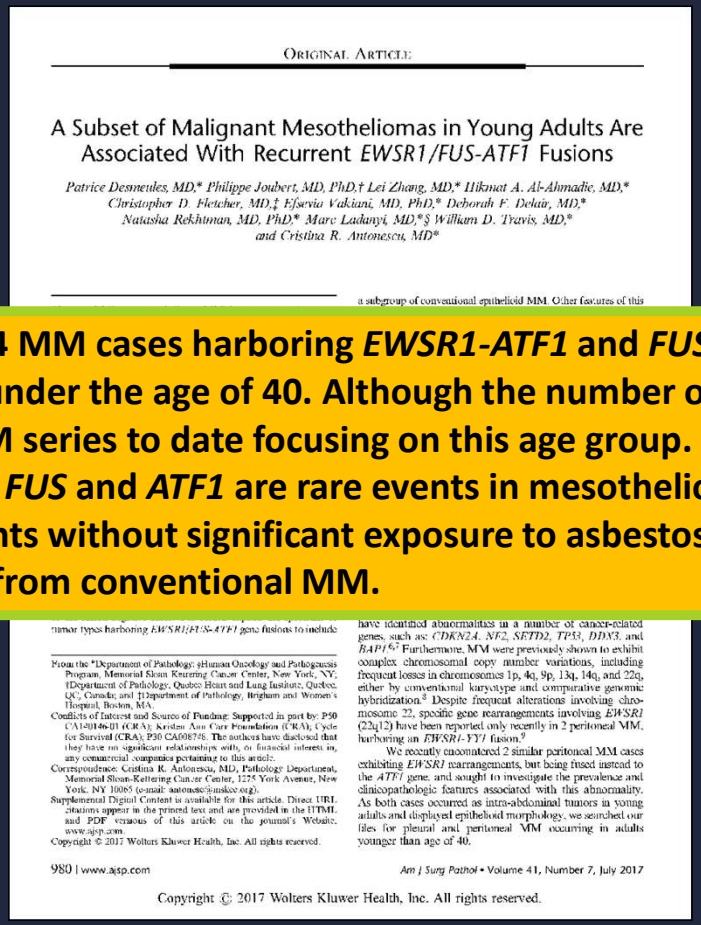

ORIGINAL ARTICLE

A Subset of Malignant Mesotheliomas in Young Adults Are Associated With Recurrent *EWSR1/FUS-ATF1* Fusions

Patrice Desmeules, MD, Philippe Joubert, MD, PhD,† Lei Zhang, MD,* Hikmat A. Al-Ahmadie, MD,* Christopher D. Fletcher, MD,‡ Efsevia Vakiani, MD, PhD,* Deborah F. Deluca, MD,* Natasha Rekhtman, MD, PhD,* Marc Ladanyi, MD,*§ William D. Travis, MD,* and Cristina R. Antonescu, MD**

In summary, we identified 4 MM cases harboring *EWSR1-ATF1* and *FUS-ATF1* fusions from a large cohort of 25 MM patients under the age of 40. Although the number of positive cases is relatively small, this is the largest MM series to date focusing on this age group. … our findings suggest that fusions involving *EWSR1* or *FUS* and *ATF1* are rare events in mesothelioma, which appear to be restricted to younger patients without significant exposure to asbestos or *BAP1* germline or somatic loss, and indistinguishable from conventional MM.

Desmukes et al. Am J Surg Pathol 2017;41:980–988.

Peritoneal Mesos in Women – Challenging Mesothelioma Causation

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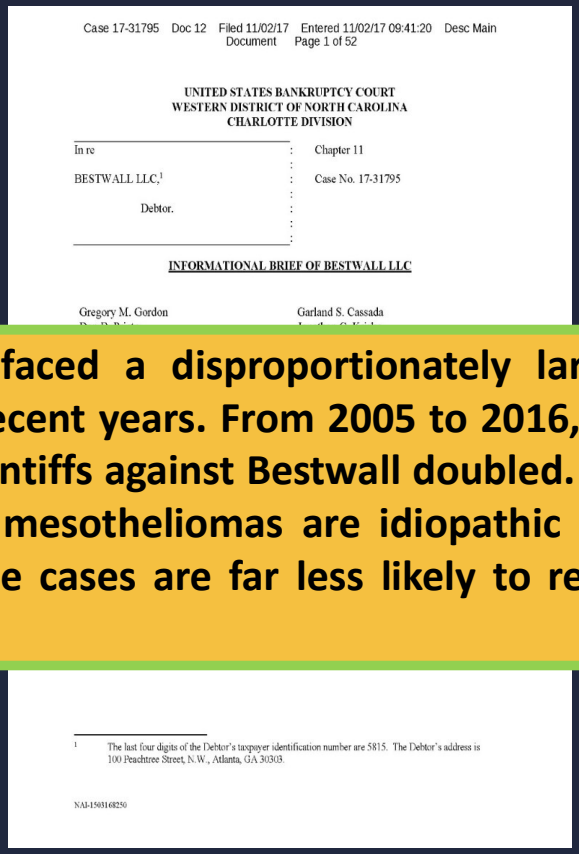

Case 17-31795 Doc 12 Filed 11/02/17 Entered 11/02/17 09:41:20 Desc Main Document Page 1 of 52

UNITED STATES BANKRUPTCY COURT
WESTERN DISTRICT OF NORTH CAROLINA
CHARLOTTE DIVISION

In re	:	Chapter 11
BESTWALL LLC,[1]	:	Case No. 17-31795
Debtor.	:	

INFORMATIONAL BRIEF OF BESTWALL LLC

Gregory M. Gordon — Garland S. Cassada

[1] The last four digits of the Debtor's taxpayer identification number are 5815. The Debtor's address is 100 Peachtree Street, N.W., Atlanta, GA 30303.

NAI-1503168250

"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."

"Informational Brief" filed by Bestwall/Georgia Pacific on 11/2/17 (Footnote 35)
https://www.scribd.com/document/379701100/Bestwall-Georgia-Pacific-chapter-11-Doc-12c-Informational-Brief-as-Filed

Recent Efforts to "Fingerprint" Asbestos-Induced Mesothelioma

- **Past and current papers show efforts to "fingerprint" asbestos induced cancers using biomarkers**
- Recent study identified four serum miRNAs (i.e. *miR-126*; *miR-205*, *miR-222* & *miR-520g*) that were shown to be directly implicated in asbestos-induced malignant diseases (*i.e.* mesothelioma and lung cancer) [Santarelli *et al*., 2018]
  - These miRNAs are well known to be involved in major pathways linked to cancer initiation and development/tumorigenesis
- Authors concluded that the discovery of a miRNA panel for asbestos-induced malignancies could have great medico-legal impact
- Limitations of the study: relatively small study population; difficult to estimate asbestos exposure

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Panou, Kindler *et al*. Identified Multiple Inherited Germline Mutations in Persons With Mesothelioma

- Multiple germline mutation study reported in paper by Panou, Kindler and colleagues, published in mid- August 2018 in the *Journal of Clinical Oncology*.
- Study showed inherited germline mutations were present in cancer associated genes **in 12% of 198 persons afflicted by mesotheliomas**
- Overall, **24 different germline mutations were identified in 13 different cancer-associated genes**
- Mutations more frequently found in persons with peritoneal mesotheliomas and/or little or no known asbestos exposure
- The subjects of the study were mainly persons treated at the University of Chicago during 2016-17

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Genetically Engineered Mouse Models (Knockout Mice)

Knocking Out Multiple Genes Induces Spontaneous Mesothelioma (In the Absence of Asbestos)

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Study Year	Multiple Genes Knocked Out (Producing Mesothelioma)
2008	*ND2*; *P53*; *INK4A*
2014	*TSC1*; *TP53*
2015	*NF2*; *INF4A*; *ARF*; *BAP1*
2018	*PTEN*; *P53*
2018	*NF2*; *CDKN2A*; *BAP1*
2019	*NF2*; *CDKN2A*; *BAP1*

Knocking Out *BAP1* Only Also Induces Spontaneous Tumors (In the Absence of Asbestos)

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Published OnlineFirst February 19, 2016; DOI: 10.1158/0008-5472.CAN-15-3371

Tumor and Stem Cell Biology

Cancer Research

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

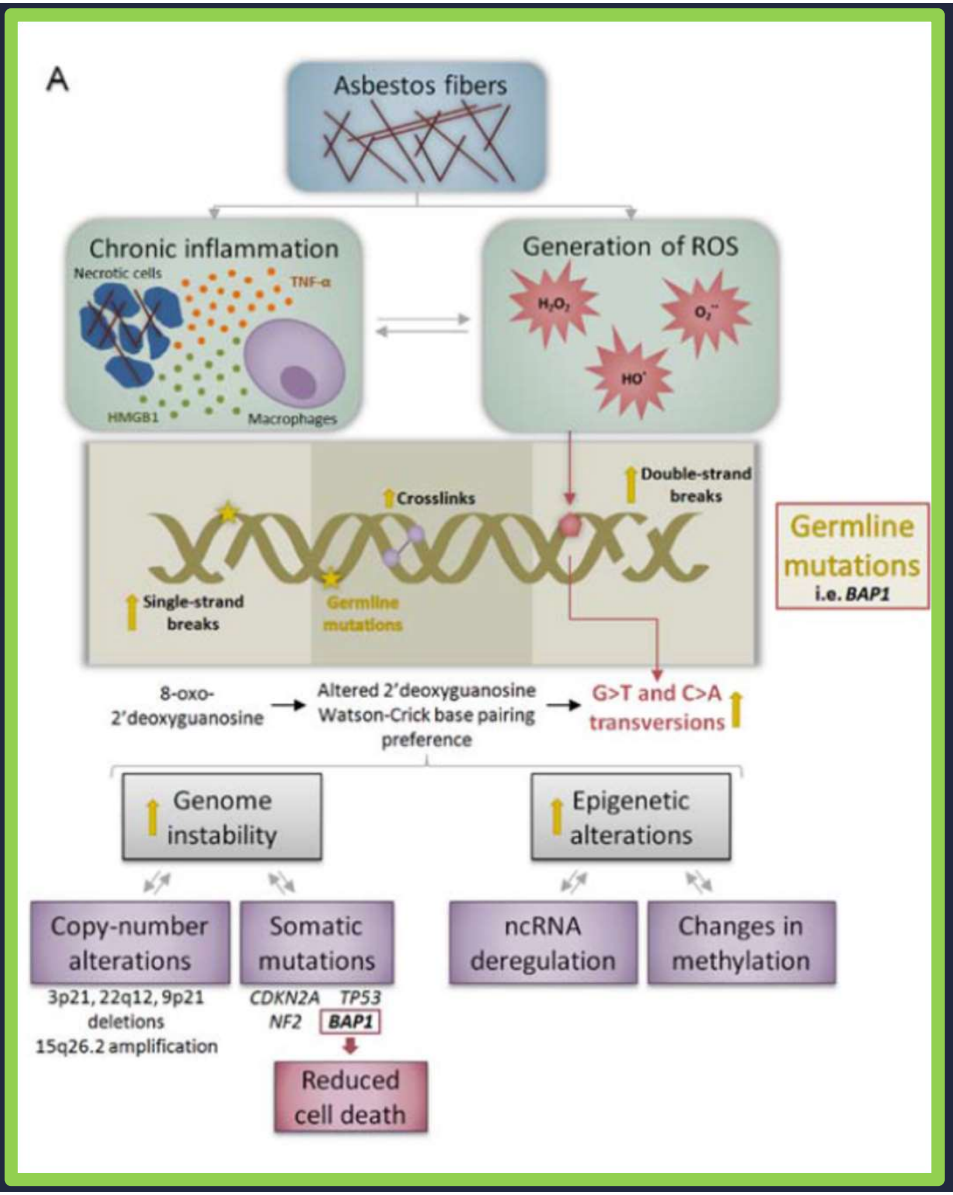
Yuwaraj Kadariya[1], Mitchell Cheung[1], Jinfei Xu[1], Jianming Pei[1], Eleonora Sementino[1], Craig W. Menges[1], Kathy Q. Cai[2], Frank J. Rauscher[3], Andres J. Klein-Szanto[2,4], and Joseph R. Testa[1]

Abstract

Individuals harboring inherited heterozygous germline mutations in BAP1 are predisposed to a range of benign and malignant tumor types, including malignant mesothelioma, melanoma, and kidney carcinoma. However, evidence to support a tumor-suppressive role for BAP1 in cancer remains contradictory. To test experimentally whether BAP1 behaves as a tumor suppressor, we

2 Spontaneous Mesotheliomas

63% Spontaneous Ovarian Cancers



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Molecular Mechanisms of Asbestos Carcinogenicity

"Asbestos-related carcinogenic effects mainly occur through two mechanisms: **activation of chronic inflammation** and **generation of reactive oxygen species (ROS)**. Both mechanisms are known to promote DNA damage in the forms of single-strand breaks, crosslinks, and double-strand breaks."

Sage *et al.*, 2018

Immune System Response to Inflammation is Intensely Individual

- **Inflammation** is a complex biological response to harmful toxicants and/or pathogens
  - Inflammation is a protective response involving our **immune cells**, blood vessels, and molecular mediators
- **Each individual will respond differently to asbestos exposure based on inherited genetics regulating immune response**
- 25%-75% per cent of our immune defense is genetically inherited/determined
  - "The strength of someone's constitution is thus genetically determined for each stimulus." University Medical Center Groningen (UMCG) and the Broad Institute of MIT and Harvard

ter Horst *et al*., 2016; Li *et al*., 2016; Aguirre-Gamboa *et al*., 2016

Genetic Susceptibility Can be Evaluated and Ruled Out or In

New and Progressing Research is Identifying Objective Genetic Markers Associated with Risk

- Presence or absence of these factors can be ruled in or out
- Review article summarizing numerous genetic variants for how a person responds to asbestos, including *CRTAM*, *SDK1*, and *RASGRF2* genes
- 10 genetic variants identified that influence how a person responds to asbestos (Matullo *et al., 2013)*
  - Further analysis of this Italian population validated existence of variants
- Caucasian population with lung cancer had microRNA variants (*MIRLET7BHG)* that *significantly* increased risk of developing lung cancer after exposure to asbestos

Genomics in Mesothelioma:
4 Key Take-home Messages

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1. Mesothelioma has an ever expanding **CAUSAL Hereditary Genetic Predisposition Component**
2. "**Genetic susceptibility**" can be evaluated and ruled out or not
3. Immune system response to **asbestos-induced inflammation is intensely individual**
4. Research groups continue seeking **molecular fingerprints for asbestos-induced disease**

More Genomic Data Will Arrive Regarding Mesotheliomas

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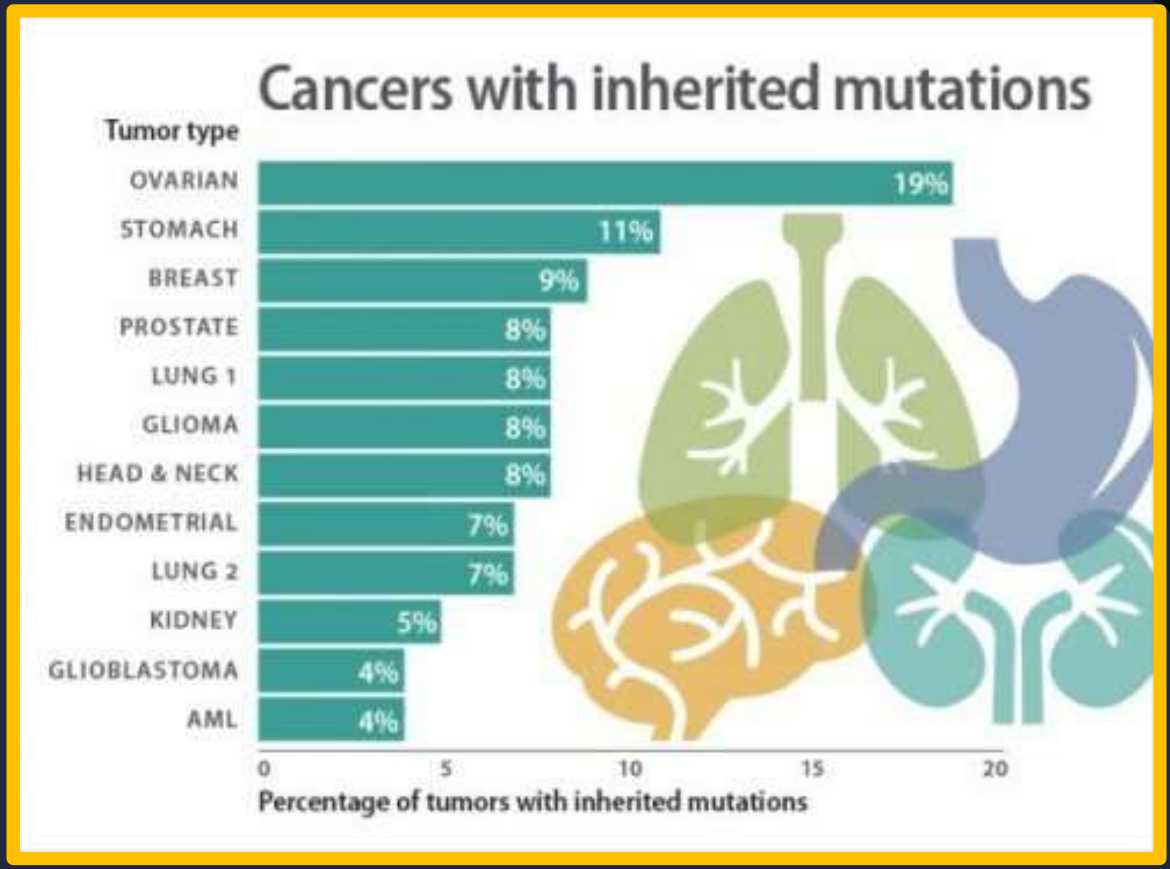
- In 2018-19, Mesothelioma Applied Research Foundation obtained Congressional funding for and
- held an initial planning meeting with CDC to begin creation of patient registry for mesotheliomas, which is intended to enhance patient care and participation in clinical trials, which in turn will lead to more data regarding pathways involved in developing mesothelioma
  - https://www.curemeso.org/get-involved/attend-conferences-and-events/symposium/patient-registry-task-force-agenda/
- Testa *et al* report they have ongoing studies with "knock in" mice with multiple germline mutations, and mice that receive injections of different types of asbestos fibers, including chrysotile fibers
- Ongoing clinical trial initiated in 2019 by NCI seeks to collect genomic data and medical outcome data for 7 years for as many as 1000 mesothelioma patients
  - https://clinicaltrials.gov/ct2/show/NCT03830229
- Fall 2019 meeting of International Association for the Study of Lung Cancer will result in multiple new publications regarding mesothelioma from prominent mesothelioma researchers

Ovarian Cancer:
Big Picture Genomic Data

Ovarian Cancer Is the Most Heritable Cancer

- Heredity is a significant risk factor for ovarian cancer
- A study published by Lu *et al.* (2015) in Nature demonstrated that ovarian cancer has the largest set of inherited germline components compared to most other cancers



https://www.nature.com/articles/ncomms10086.pdf

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

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Genetic Factors for Ovarian Cancer Go Far beyond *BRCA* Genes, 32 Known Germline Genetic Drivers for Ovarian Cancer

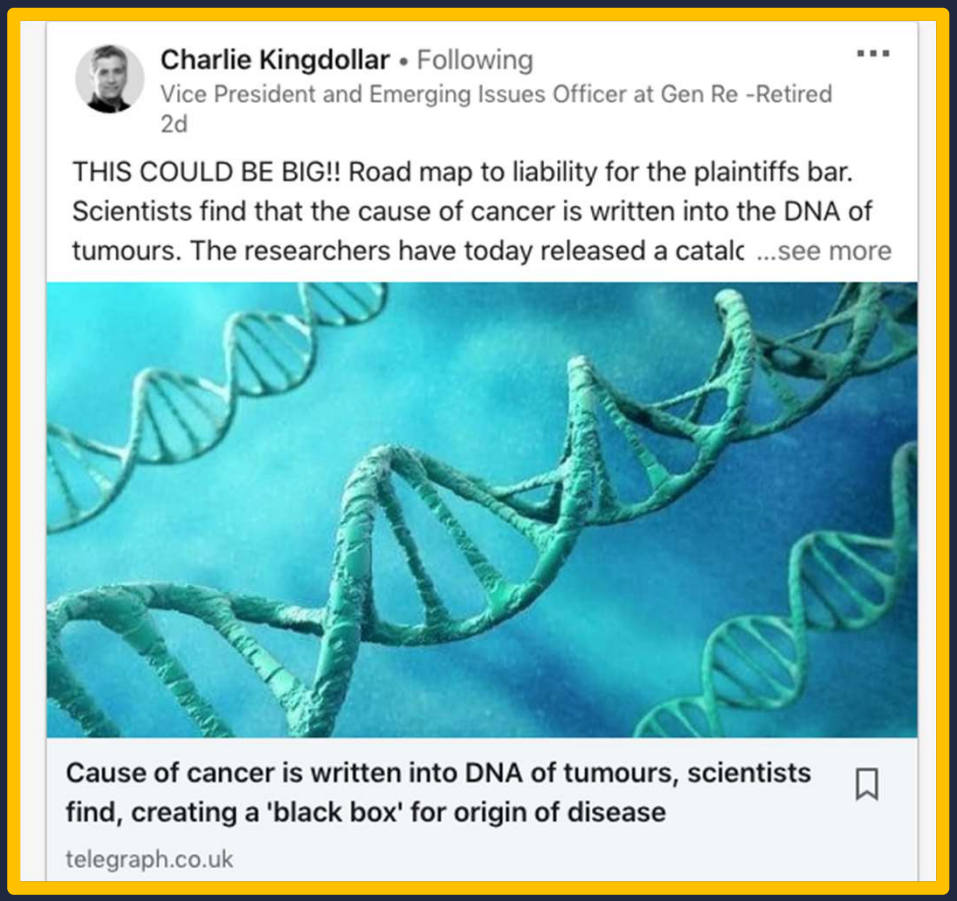
- Researchers from across the world analyzed the DNA of nearly 100,000 women with the most common types of ovarian cancer and compared them to healthy controls
- **As a result, they identified 12 new germline genetic drivers of ovarian cancer**
- They also confirmed that 18 previously identified germline variants drive ovarian cancers [Phelan *et al*., 2017, Nature Genetics 49 (5), 680-691]
- **Due to this major international study, it is known that ovarian cancers are driven by 30 germline genetic variants in addition to *BRCA1* and *BRCA2***.

Phelan *et al*., 2017, Nature Genetics 49 (5), 680-691

Somatic Variant Genetic Testing

Sequencing Tumor Cells to Find "Mutation Signatures" That Identify Causes of Cancers

Mutational Signature Papers Attracting Attention From Insurance Professionals



In our view, use of somatic signature analysis offers potential risks and benefits for both plaintiffs and defendants

An Example:
Signature Mutation Analyses to Distinguish Between Causes of Lung Cancers

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- **Plaintiff and defense lawyers will want to pay increasing attention to "mutational signature" patterns in lung cancers**.
- Multiple, respected research groups have investigated signature mutation patterns in lung cancers in smokers and non-smokers
- Somatic signature patterns for smoking induced lung cancers have been published in major scientific journals. (*e.g.* Alexandrov *et al*., 2013)

An Example:
Signature Mutation Analyses to Distinguish Between Causes of Lung Cancers

- Newer research is revealing germline mutation patterns of **genetically caused** lung cancers in non-smokers. (Lee *et al.*, 2019: Tracing Oncogene Rearrangements in the Mutational History of Lung Adenocarcinoma. Cell. 2019 Jun 13;177(7):1842-1857)
- A key quote: **"Our study highlights [lung cancer] oncogenesis driven by endogenous mutational processes."**

Asbestos:
Somatic Mutation Analysis

Asbestos-related molecular alterations in lung cancer – Helsinki at 139

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Alteration	Putative consequence or carcinogenic association	Type of study	References
AI and loss at 2p16	Cell process	Lung cancer of asbestos-exposed individuals	105
LOH at3p14	FHIT exon loss	Lung cancer of asbestos-exposed individuals	115, 116
LOH at 3p21	Possible down-regulation of tumor suppressors	Lung cancer of asbestos-exposed individuals	99, 117
LOH/homozygous deletion at 9p21.3	Loss of P16/CDKN2A	Lung cancer of asbestos-exposed individuals	108, 109
CNA at 9q33.1	Loss of DBC1	Lung cancer of asbestos-exposed individuals	111
AI and loss at 19p13	Possible down-regulation of tumor suppressors, e.g. KEAP1	*In vitro*; lung cancer of asbestos-exposed individuals	100, 112
Polyploidy	Aneuploidy and chromosomal instability	*In vitro*; lung cancer of asbestos-exposed individuals	111, 118
Up-regulation of TP53	Decreased or abnormal tumor suppressor activity possibly due to mutations	*In vitro*; lung cancer of asbestos-exposed individuals	119, 120
Serum Ras (p21)	Up-regulation due to mutations	Asbestos-exposed lung cancer patients	121
KRAS	Specific mutations	Lung cancer of asbestos-exposed individuals	122, 123

Asbestos, asbestosis, and cancer, the Helsinki criteria for diagnosis and attribution 2014: recommendations, Scand J Work Environ Health, doi:10.5271/sjweh.3462

Recent Example of Growing Literature on Somatic Signature Mutations For A Wide Range of Substances

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A Compendium of Mutational Signatures of Environmental Agents

Jill E. Kucab, Xueqing Zou, Sandro Morganella, Madeleine Joel, A. Scott Nanda, Eszter Nagy, Celine Gomez, Andrea Degasperi, Rebecca Harris, Stephen P. Jackson, Volker M. Arlt, David H. Phillips, Serena Nik-Zainal

Cell

DOI: 10.1016/j.cell.2019.03.001

Cell PRESS

A Compendium of Mutational Signatures of Environmental Agents

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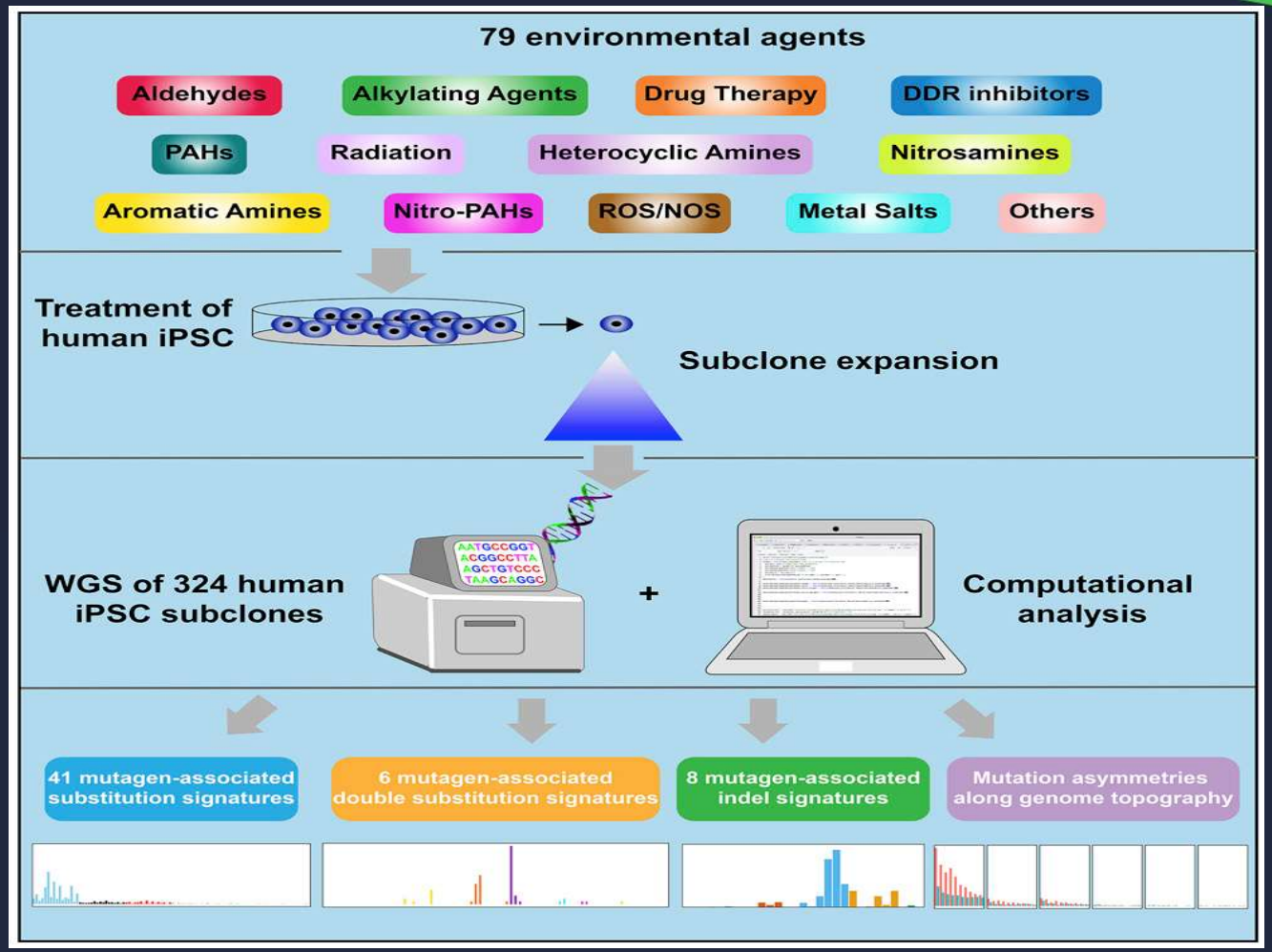
- Paper reports a study in which stem cells were dosed with 79 different environmental agents
- Whole genome sequencing were then used to look for mutational signatures within the cells
- 41 of 79 environmental agents produced somatic signature mutation patterns
- Many of the patterns found are statistically significant at the .01 or .001 level
- Some of the somatic signature patterns found in cell lines are similar to somatic mutation patterns found in prior studies of tumors in humans with known exposures to a particular environmental agents of interest

Kucab *et al*., 2019 – Cell – online April 11, 2019



Cell DOI: (10.1016/j.cell.2019.03.001)

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Multidisciplinary group of geneticists, scientific consultants, and counsel offering consulting and expert services in genomics & systems biology regarding toxins

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- GlobalTort blog - https://www.globaltort.com/
- Innovative Science Solutions blog - http://www.innovativescience.net/blog/

Disclaimer

- Presentation slides are provided for educational and informative purposes, with the caveat that they are condensed and imperfect methods for communicating sometimes complex information

- The individual and collective slides are not intended to cover all factors that may be relevant to a particular situation, and should be considered in context

-

Supplemental Materials

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Supplemental Materials on Precision Medicine

What is Precision Medicine?

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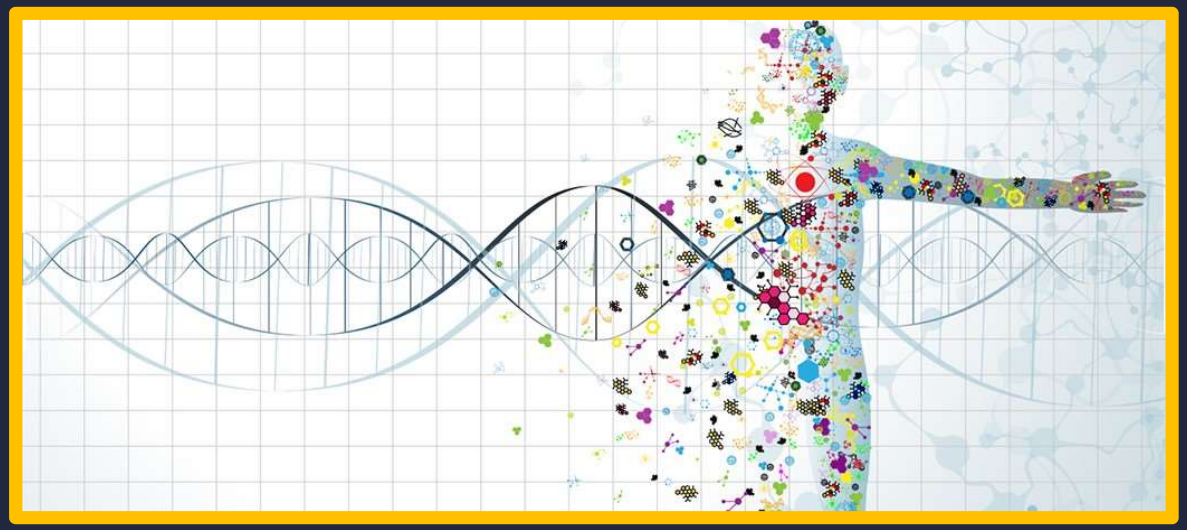
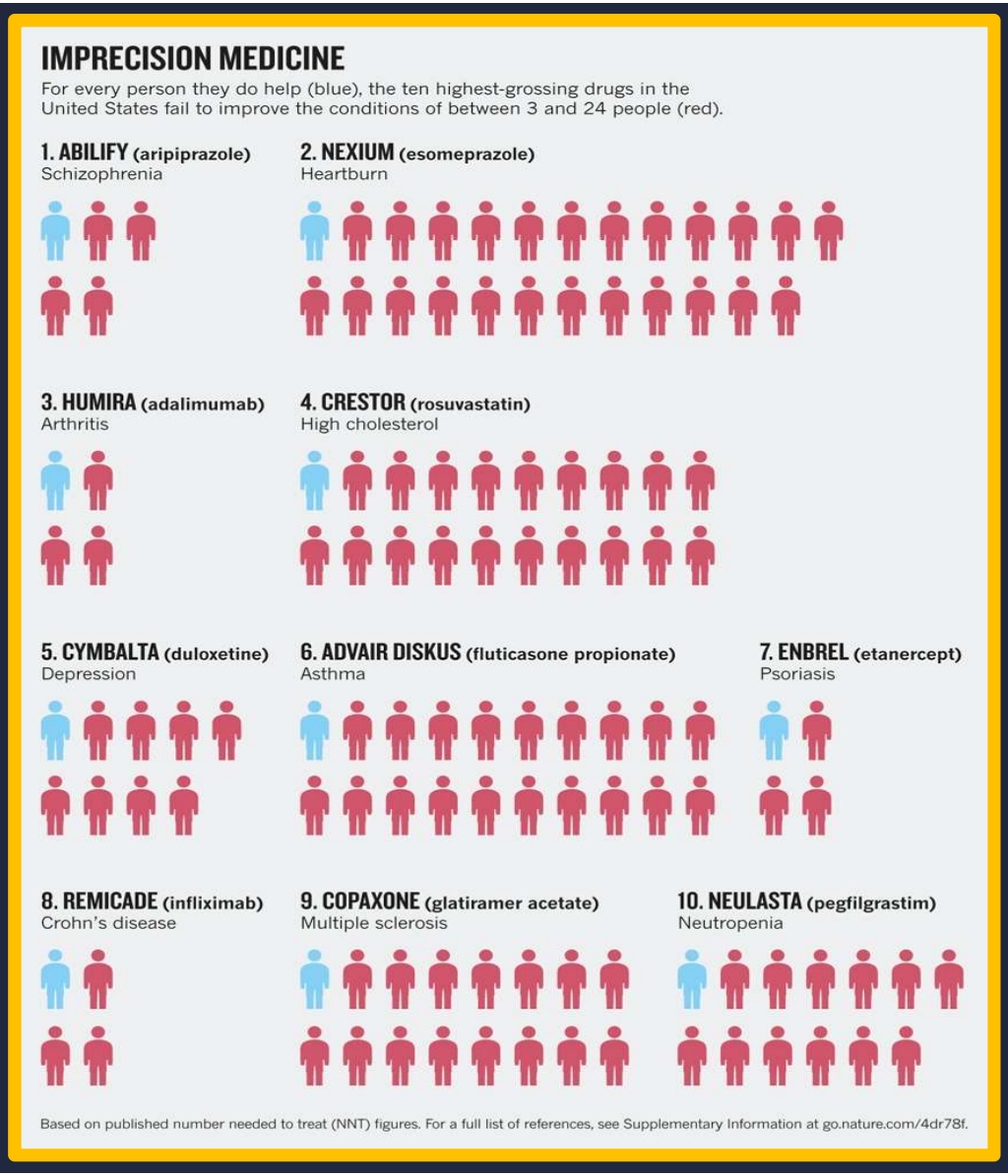
Precision medicine is a model of clinical care **that accounts for the individual variability in genes, environment, and lifestyle of each patient**.



https://www.nih.gov/precision-medicine-initiative-cohort-program



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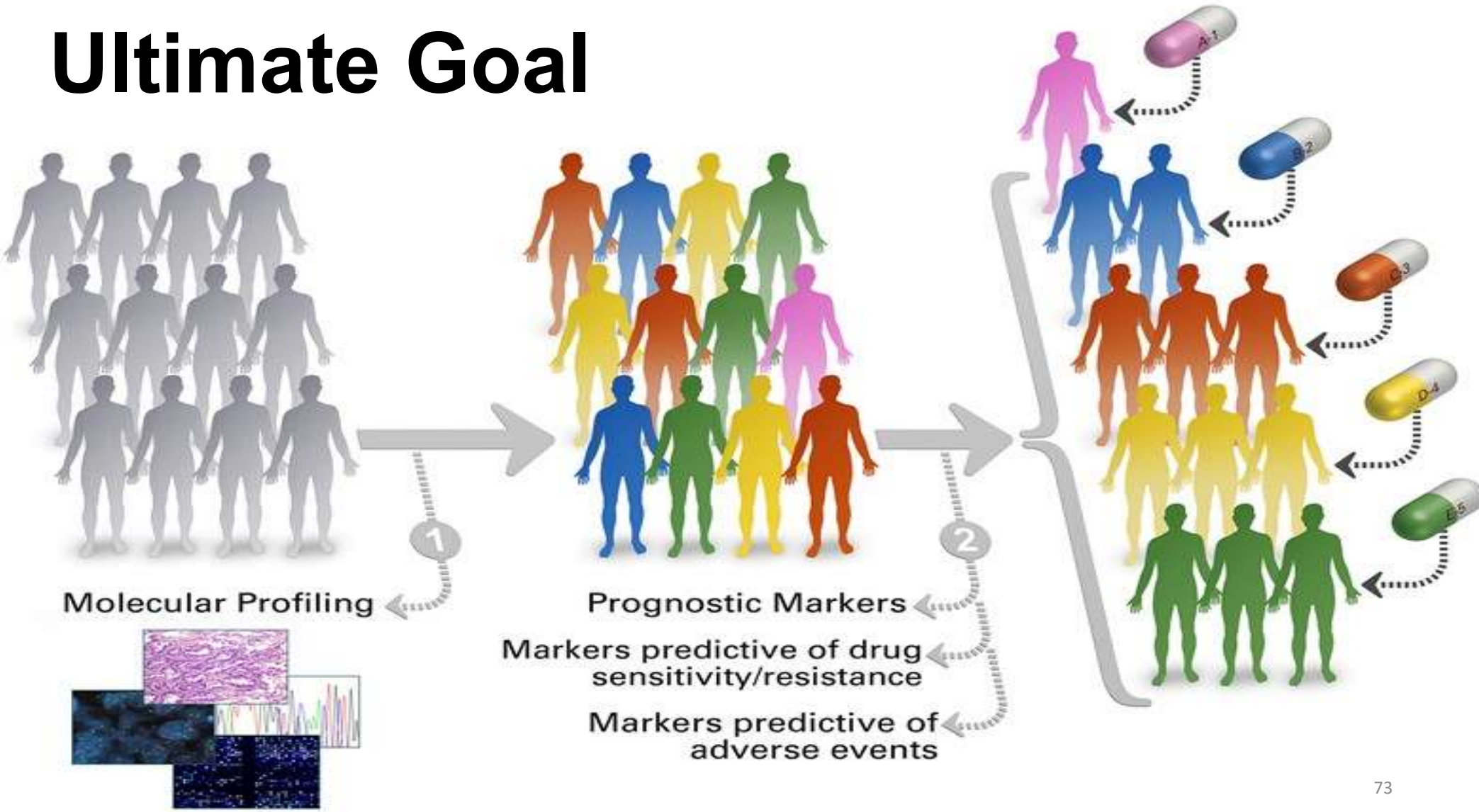
Population Medicine

Only 4-33% of patients experience improved health outcomes from the 10 highest grossing commercial drugs. The remaining **67-96% DO NOT BENEFIT!**

Schork, 2015

Ultimate Goal



A ‘Google Maps’ -like Knowledge Network for Precision Medicine

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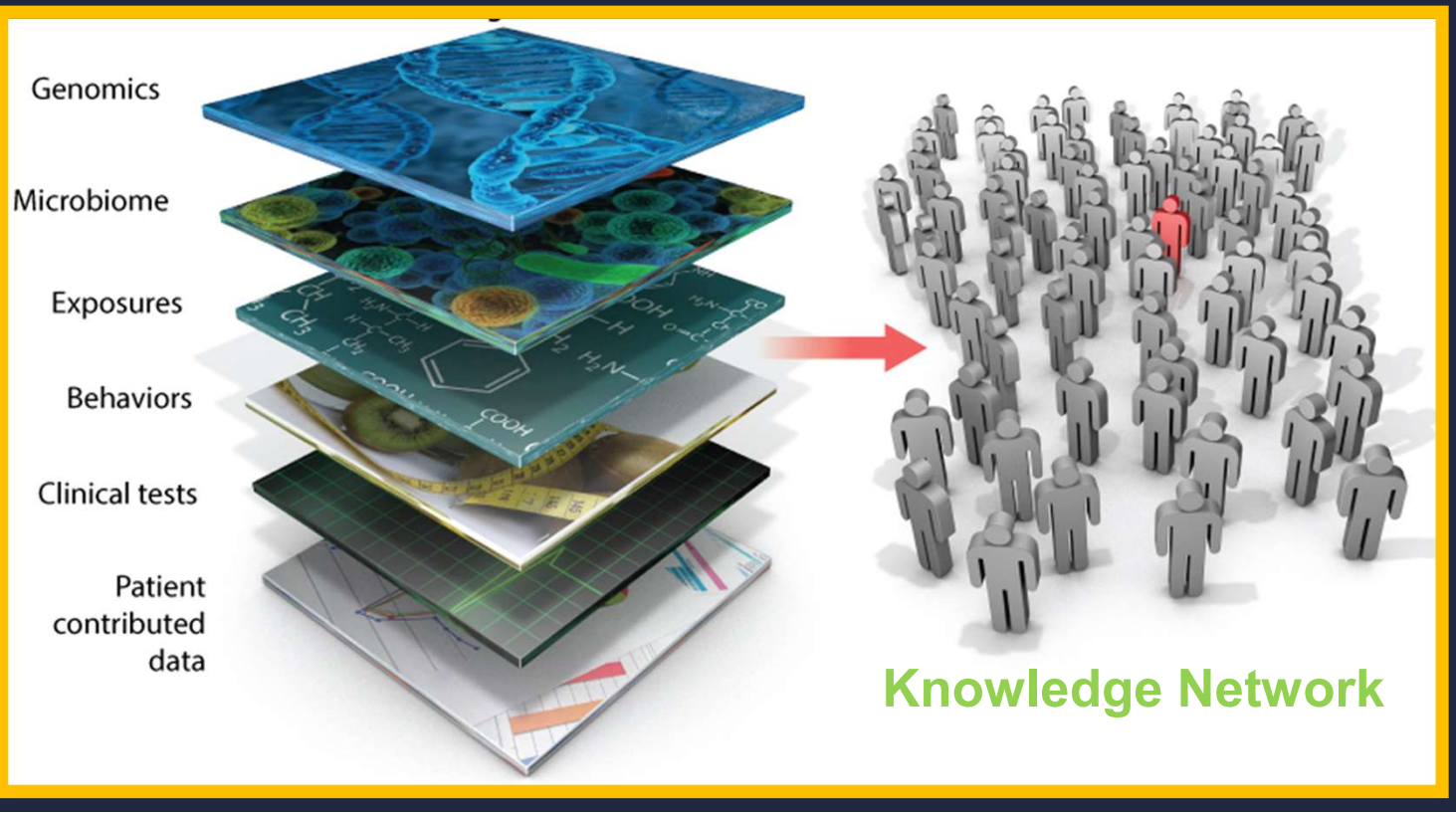

- Defines disease mechanisms
- Drives drug development
- Informs prevention and diagnosis-therapy decisions for individuals

Since the 1990s: Mortality Down, Survivorship Up!

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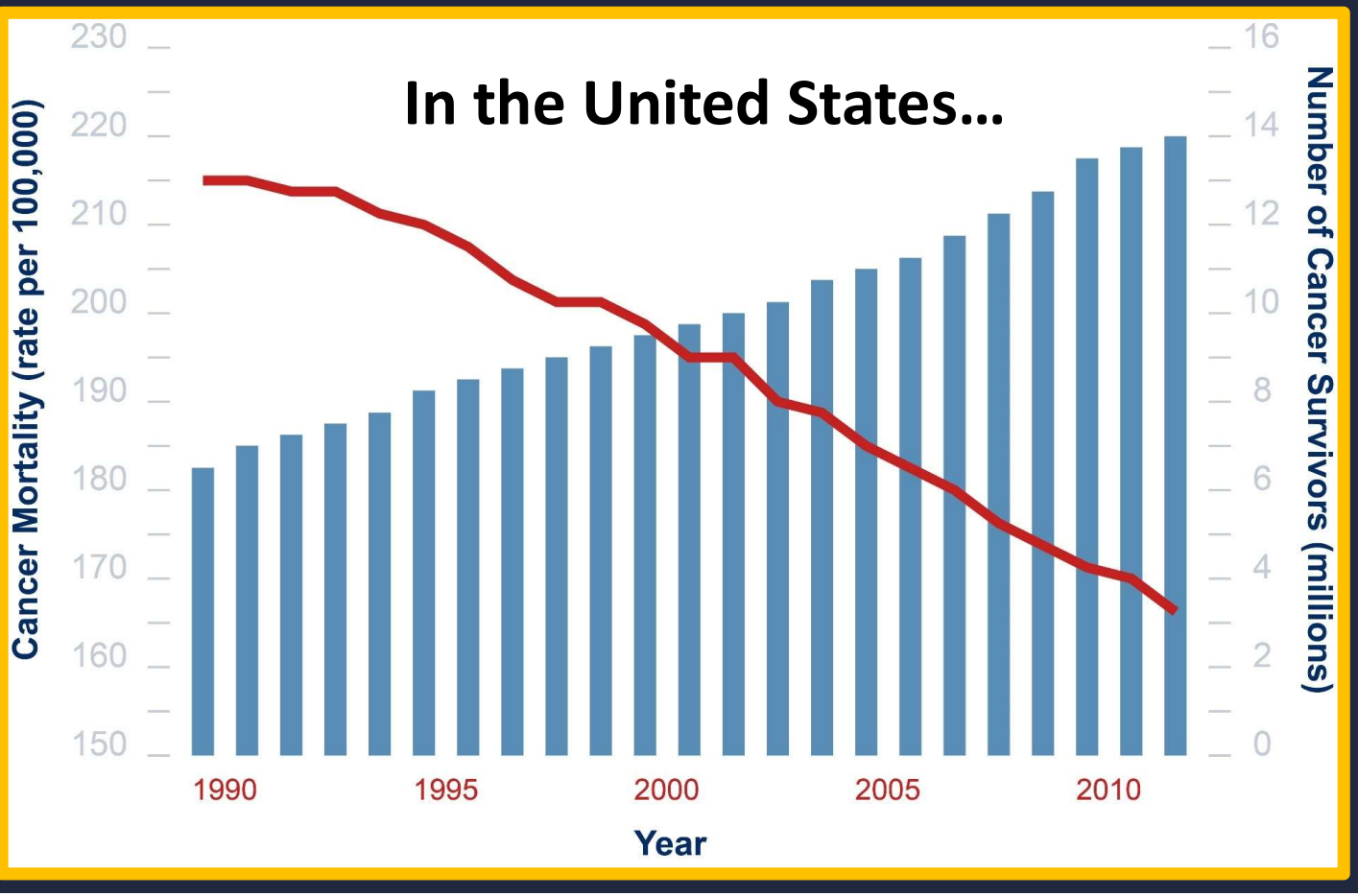
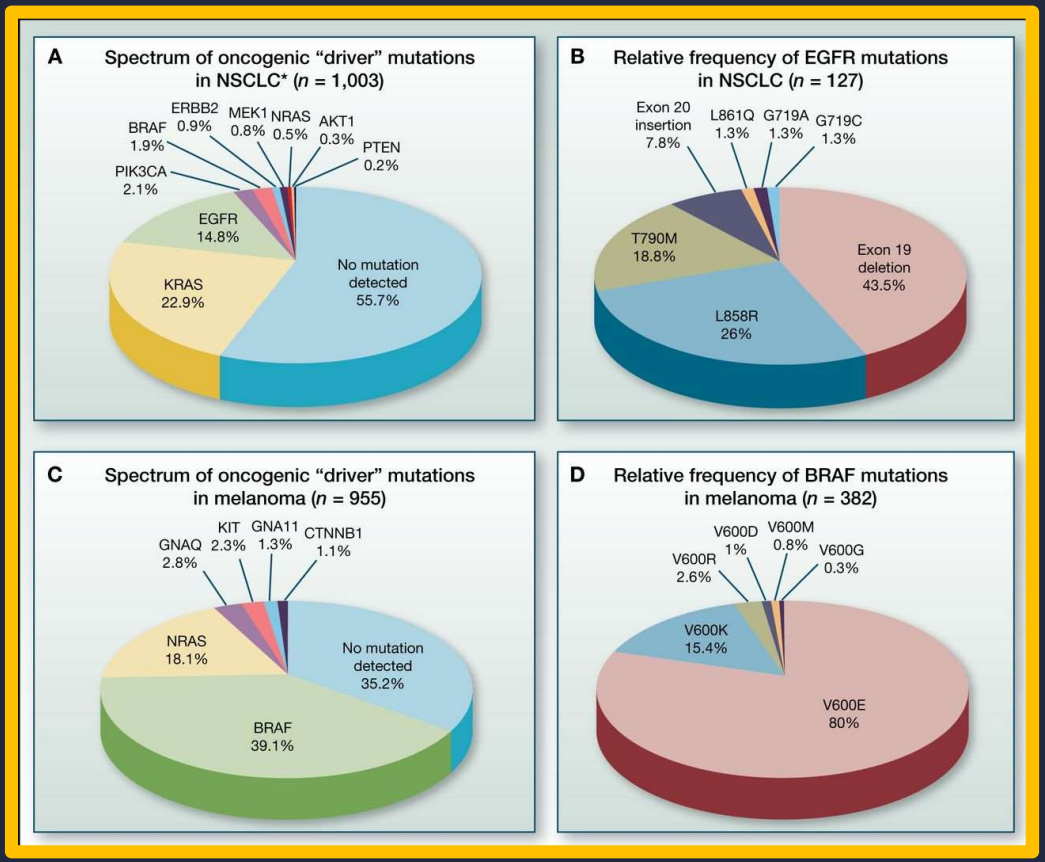


Sources: US Mortality Files, National Center for Health Statistics, CDC. DeSantis C, Chunchieh L, Mariotto AB, et al. (2014). Cancer Treatment and Survivorship Statistics, 2014. CA: A Cancer Journal for Clinicians.

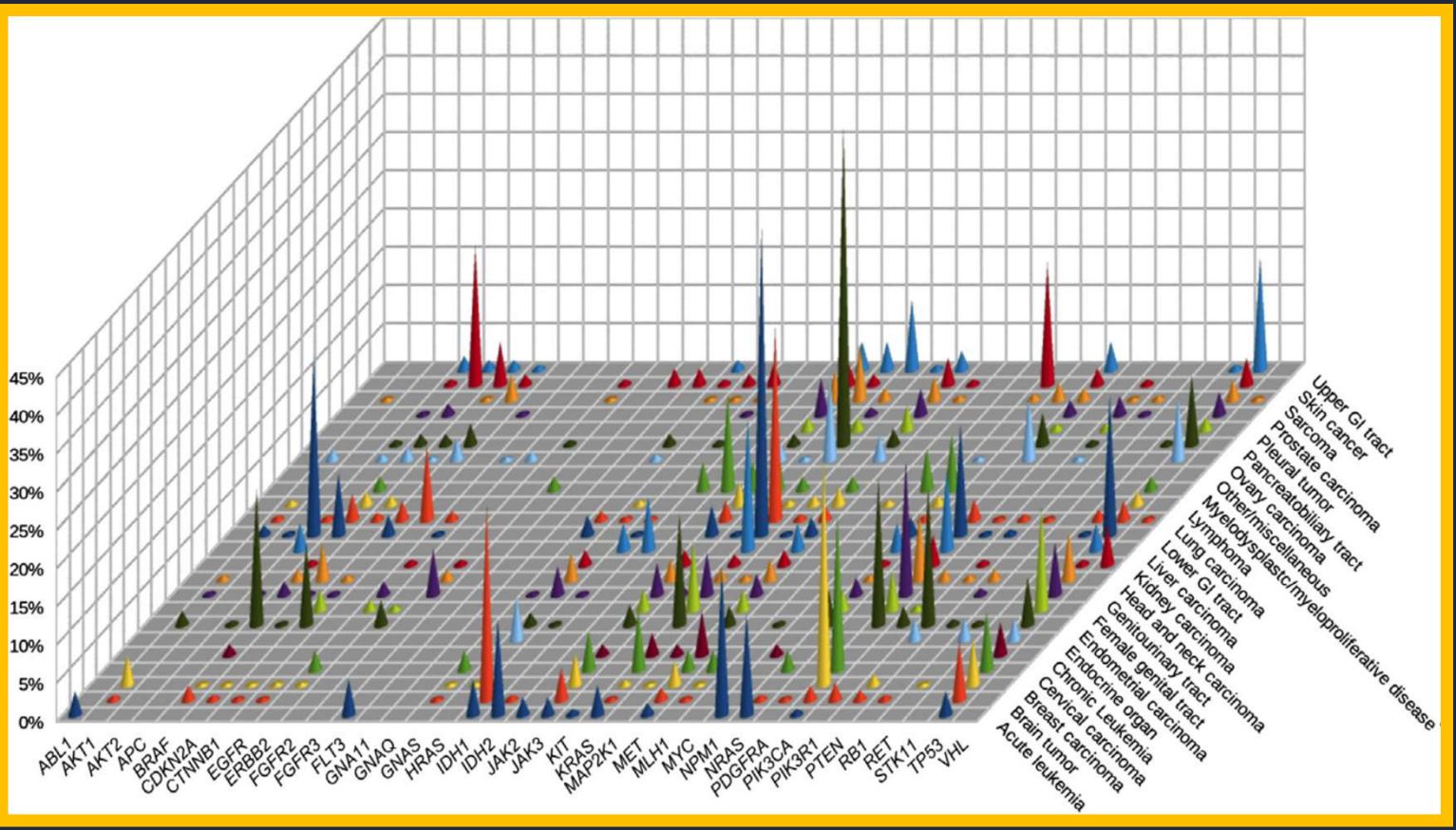
Supplemental Materials on Mutational Patterns in Cancers

Common Cancers are Now Collections of Rare Cancers



Meador *et al.*, 2014

Genomic Landscape of Human Cancers



MacConaill *et al*., 2014

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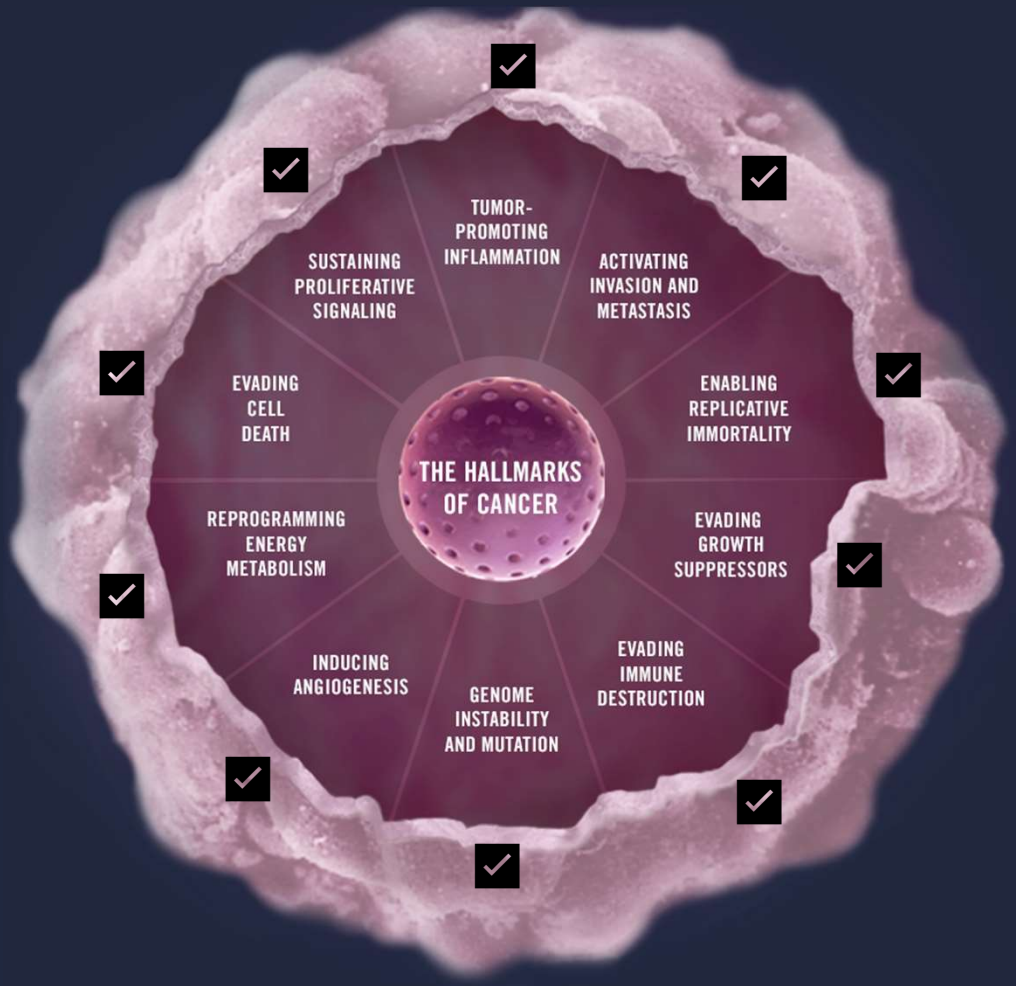
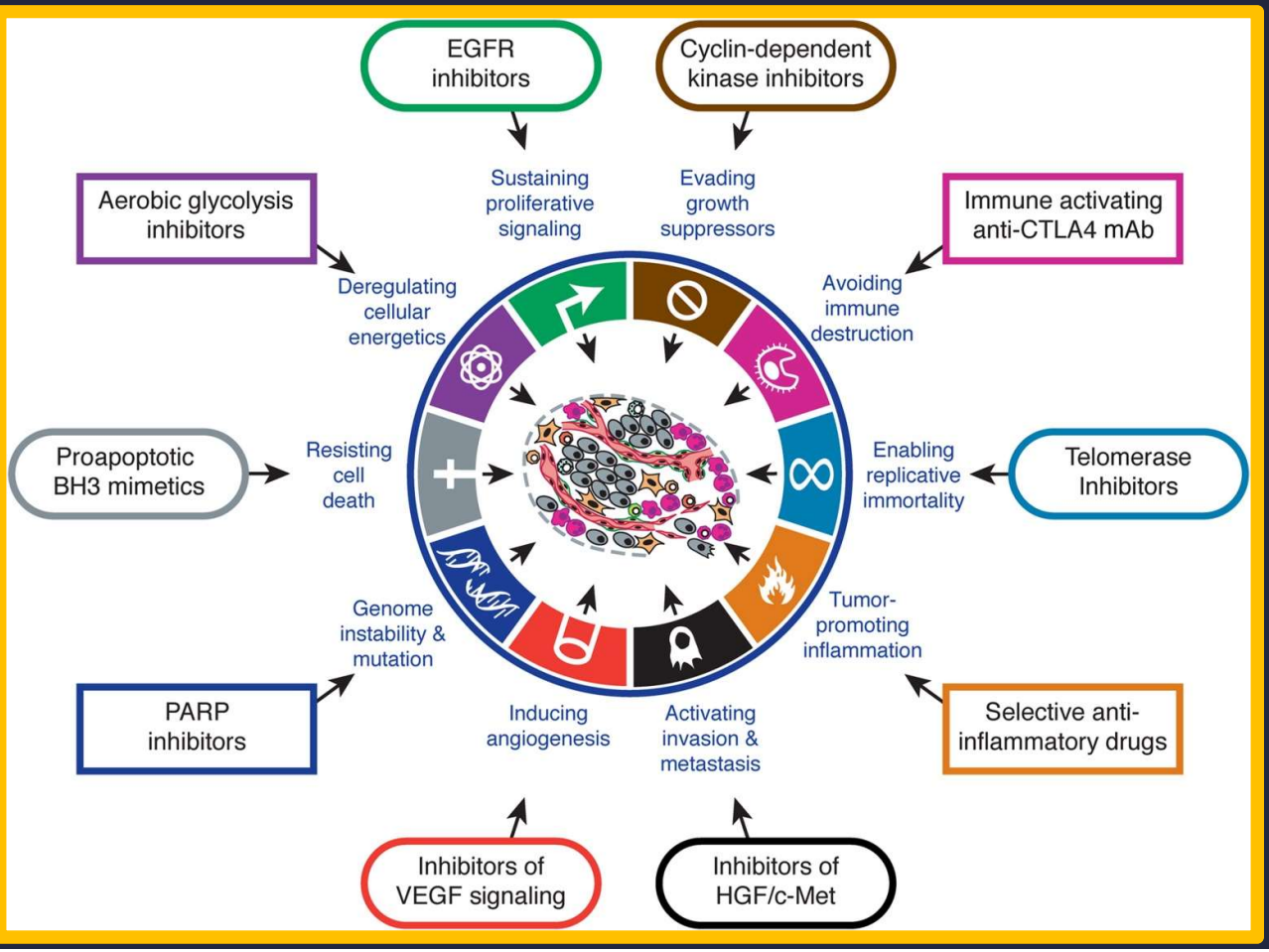


"Hallmarks of Cancer"

Cell PRESS

Cell 2000 100, 57-70DOI: (10.1016/S0092-8674(00)81683-9)

Hallmarks of Cancer: Therapeutic Implications



Drugs that interfere with each of the acquired capabilities necessary for tumor growth and progression have been developed and are in clinical trials or in some cases approved for clinical use in treating certain forms of human cancer.

Cell PRESS

Cell 2011 144, 646-674DOI: (10.1016/j.cell.2011.02.013)