Genetic Biomarkers of Risk in Toxic Tort Litigation

Gary Marchant, Arizona State University
Kirk Hartley, LSP Group LLC
The Genetic Era is (Finally) Here
Genetics and Toxic Torts

• Two major types of data:
  • Genetic susceptibility to toxic agents
  • Genetic biomarkers of exposure or effect
• Can benefit plaintiffs or defendants in appropriate cases
  – Like forensic DNA in criminal cases, genetic biomarkers in tort cases have the potential to inculpate the guilty and exonerate the innocent
• Doctrinal templates for using genetic data in toxic torts already exist
Two Case Studies

Benzene – Gary Marchant
Asbestos – Kirk Hartley
Example One: Benzene

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Benzene and Leukemia: Chromosomal Biomarkers

- A number of cases where parties argue that specific chromosomal aberration is probative of benzene-caused leukemia
  - e.g., translocations between chromosomes 5 and 7 in acute myleogenous leukemia (AML) from benzene
  - e.g., translocations between chromosome 15 and 17 in acute promyelocytic leukemia (APL) from benzene
- Presence or absence of such translocations can be used by plaintiffs or defendants to argue for/against specific causation
Biomarkers of Effect: Absence of Causation

- Presence or absence of a characteristic genetic biomarker evidence for/against causation
- *Wells v. Shell Oil Co.*
  - Worker alleged that he contracted acute myelogenous leukemia (AML) from benzene
  - Defendant successfully argued that benzene can only produce AML by breaks in chromosomes five and seven; argument rejected in two subsequent cases
- Plaintiffs have also argued (unsuccessfully) that presence of specific chromosomal aberration involving chromosomes 5 and 7 argues in favor of causation
Milward v. Acuity Specialty Products Group

• Plaintiff exposed to benzene suffered from Acute Promyelocytic Leukemia (APL) which apparently always involves translocation involving chromosomes 17 and 15
• Plaintiff’s expert (Martyn Smith), as part of “weight of evidence” approach, argued that because benzene known to cause other chromosome breaks (eg 5, 7 breaks), it is plausible it could cause 15,17 break characteristic of APL
• District court (Mas.. 2009) held that a paper co-authored by Dr. Smith concluded that “benzene can initiate or promote leukemia induction by a nonrandom selective effect” on specific chromosomes, and this defeated “the generalization that because … benzene causes damage to some chromosomes, it is ‘biologically plausible’ that it causes damage to other chromosomes.”; held Smith testimony held to be non-admissible
• 1st Circuit overturned decision – held that district court “both placed undue weight on the lack of general acceptance of Dr. Smith’s conclusions and crossed the boundary between gatekeeper and trier of fact”
  – “There is an important difference between what is unreliable support and what a trier of fact may conclude is insufficient support for an expert’s conclusion.”
## Benzene Biomarkers: Plaintiff v. Defendant

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Benzene Biomarkers: Judge vs. Jury

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Biomarkers of Effect: Supporting Specific Causation

  – Plaintiff smoker had adenocarcinoma; claimed tobacco smoke caused his tumor
  – Key evidence – plaintiff’s expert testified that deletions in 3 specific chromosome regions with tumor suppressor genes
    • Data suggested that each of these deletions is more common in smokers with adenocarcinoma than in non-smokers with adenocarcinoma
  – Court upholds admission of testimony
By detecting early changes in gene expression one can also identify the “molecular signature” of acute benzene poisoning.

Bruce Gillis, Cytokine Institute
Identification of human cell responses to benzene and benzene metabolites

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Abstract

Benzene is a common air pollutant and confirmed carcinogen, especially in reference to the hematopoietic system. In the present study we analyzed cytokine/chemokine production by, and gene expression induction in, human peripheral blood mononuclear cells upon their exposure to the benzene metabolites catechol, hydroquinone, 1,2,4-benzenetriol, and p-benzoquinone. Protein profiling showed that benzene metabolites can stimulate the production of chemokines, the proinflammatory cytokines TNF-α and IL-6, and the Th2 cytokines IL-4 and IL-5. Activated cells showed concurrent suppression of anti-inflammatory cytokine IL-10 expression. We also identified changes in global gene expression patterns in response to benzene metabolic challenges by using high-density oligonucleotide microarrays. Treatment with 1,2,4-benzenetriol resulted in the suppression of genes related to the regulation of protein expression and a concomitant activation of genes that encode heat shock proteins and cytochrome P450 family members. Protein and gene expression profiling identified unique human cellular responses upon exposure to benzene and benzene metabolites.

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Keywords: Benzene; Hydroquinone; 1,4-Benzoquinone; 1,2,4-Benzotriol; Catechol; Cytokines; Chemokines; Gene expression profiling; Immune response

Benzene is a widely used industrial chemical and one of the most common air pollutants, with emissions reaching 7 million pounds annually in the United States [1]. The role of benzene in the causation of human health effects has long been well established and this is especially so regarding its carcinogenic properties. It is a contaminant that is a by-product of petrochemical processes; vehicular engine exhaust, and cigarette smoking, among other sources. It was among the first confirmed carcinogens established by the International Agency for Research on Cancer [2]. The roles of benzene and benzene metabolites in human health have centered around toxic and injurious effects to the hematopoietic system, including but not limited to bone marrow suppression, particularly in reference to acute and chronic leukemias (reviewed in [3]). Other associations have been acknowledged, including in regard to lymphomas, myelodysplastic syndrome, multiple myeloma, and malignant melanoma [2].

It is generally agreed that the toxicity of inhaled benzene results from its biotransformation to reactive species. Benzene is metabolized in the liver by cytochrome P4502E1 (CYP2E1) to phenol, which undergoes subsequent hydroxylations to hydroquinone, catechol, and 1,2,4-benzenetriol [4]. Catechol is further oxidized to 1,4-benzoquinone by bone marrow peroxidases or by autoxidation. The intermediate benzene epoxide can also undergo ring opening to trans-muconic acid. Because many reactive metabolites are formed during benzene metabolism, it is likely that benzene toxicity is mediated through multiple pathways.

Benzene-induced leukemia is unique because benzene and its metabolites do not have the same DNA-binding properties as "classic carcinogens" [5]. Different mechanisms for benzene-induced toxicity leading to neoplasia have been proposed [6].
Legal Exposure

Attorneys look to Cytokine Institute's chemical analysis for guidance in lawsuits

By BETH COWLEY

The Cytokine Institute has confirmed its test results on a series of chemicals, leading to the settlement of several lawsuits.

The institute's testing has been praised by plaintiffs' attorneys as a significant force in the litigation process. Despite challenges from the chemical industry, the institute has continued to provide credible evidence for those seeking justice.

Cytokine Institute LLC

Founded: 1995

Gene Business: Laboratory testing for toxicology

Employees: 30

Cytokine testing methods for environmental monitoring

Beverage Testing: Determining chemical compounds in beverages

Gary R. Rupp, President

The Cytokine Institute has confirmed its testing methods are reliable and accurate, providing a valuable resource for legal cases in the field of toxicology.
Commentary

Misuse of Genomics in Assigning Causation in Relation to Benzene Exposure

MARTYN T. SMITH, PHD

Benzene is an established cause of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), and may also cause lymphocytic leukemias and non-Hodgkin lymphoma (NHL) in humans. Additionally, changes in blood and bone marrow consistent with hematotoxicity are recognized in humans and experimental animals. Despite extensive research, questions remain regarding the exact mechanisms by which benzene and/or its metabolites exert their observed health effects; novel biomarkers of exposure and relevant early biologic effects are needed. Biomarkers or medical tests which could demonstrate past exposures responsible for benzene-
civil courts in California have already heard more than 20 cases that used evidence from the technique. In one case, a worker at a company selling tyres sued his employers alleging that he had suffered illness as a result of exposure to benzene. Liberty Mutual, the employer’s insurers, paid for the test to be carried out which proved the illness was not caused by the chemical, saving an estimated $1million in damages.

Having studied the toxicology of benzene for many years, I was not sure how such a technique could possibly work, so I investigated further. I soon learnt that the basis for the test was an article in the journal Genomics

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‘Details of the msds1TM test are unclear but the Cytokine Institute web site claims it “relies on no less than 22,000 DNA-based parameters” ... [T]here is no possibility that it can reliably help us assign causation in relation to benzene exposure ... [T]he msds1TM test is clearly junk science. Apparently, this supposed genomic technology has been uncritically accepted by both the workers’ compensation bar and has been used to deny workers compensation benefits without scientific challenge. This acceptance has occurred despite the fact that the msds1TM test has never been subject to an analysis of sensitivity, specificity or positive predictive value. No knowledgeable scientist would accept the msds1TM test as useful information in attributing disease causation. Unfortunately the msds1TM test, though not a scientific breakthrough, may represent a new advance in “blinding people with science,” a colloquial British expression meaning to deliberately confuse someone by giving the impression of highly complex knowledge.’
Biomarkers To Prove Exposure

• “[T]here are biological tests (biomarkers) that measure the levels of chemicals in the body to reveal whether these levels can exceed expected or accepted levels. .... [B]ecause no such tests were performed on Mr. Cord, ‘it is impossible to determine to a medical certainty’ whether Mr. Cord's exposure, absorption or toxicity to benzene or other chemicals exceeded normal and expected levels. In other words, existing tests were available to measure whether Mr. Cord in fact had excessive exposure to benzene and other chemicals, but plaintiffs' experts did not use them.” Cord v. City of Los Angeles (Cal. App. Sept. 30, 2004).
Biomarkers: Exposure

  – Spouse of lab tech who died of multiple myeloma claimed benzene exposure caused her husband’s illness and death
  – “Decedent’s blood tests, taken after his alleged exposure from 1977 to 1982, ... showed no evidence of benzene exposure” and thus “judge concluded that decedent did not prove sufficient exposure to benzene ....” (quotations omitted)

  – Plaintiff claimed husband’s occupational exposure to benzene caused AML
  – Plaintiff’s expert testified blood of victim had chromosomal aberrations that were characteristic of benzene exposure; court held that this evidence did not relieve plaintiff of duty to demonstrate exposure
Example Two:
Asbestos

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Germline Mutations and Asbestos Litigation
Mesothelioma - Familial Mutation Identified

• BAP1 germline mutation familial cancer syndrome identified in papers in 2011

• BAP1 = BRCA associated protein # 1

• Several cancers involved in the syndrome, including eye and skin cancers, as well as mesotheliomas

• Germline BAP1 mutations predispose to malignant mesothelioma.
    • Nat Genet. 2011 Aug 28;43(10):1022-5. doi: 10.1038/ng.912

• Trial courts are starting to receive and rule on motions for germline testing

• Two rulings to date (1 not yet final) allow testing but require destruction of data at the end of the case, at the request of counsel for plaintiffs
  – Rulings requiring destruction reflect lack of good argument, lack of judicial knowledge, and fairly common trial judge instinct to simply manage the narrow issue at hand instead of taking a broader view
Testing for Mesotheliomas in Mice Engineered for BAP1

• One of the authors of BAP1 papers (Testa) is involved in ongoing testing of BAP1 mice for extent of BAP1 impacts and mesotheliomas with and without asbestos exposure

• June 2014 paper by Testa and colleagues after peritoneal injections of crocidolite fibers into mice with heterozygous BAP1 mutation

• Crocidolite asbestos exposure - plus the BAP1 mutation – “accelerates the development of mesothelioma”

• No mesos observed in controls

• But paper predated natural death or sacrifice of all mice – changes are possible

• Studies and grant ongoing through 2018
• University of Pennsylvania: Superfund Research Program
  – Project Leader: Joseph R. Testa (Fox Chase Cancer Center)
    Grant Number: P42ES23720
    Funding Period: 2014-2018
BAP1 Researchers Disagreeing in Court on “Causation”

- Two BAP1 researchers (Testa and Carbone) recently disagreed in affidavits in court on significance of BAP1 as to causation with and without exposure to asbestos fibers

- Testa - Para 31 - “I have reviewed the "Declaration of Michele Carbone, M.D. in Support of CertainTeed's Motion for Blood Sample and Limited Genetic Testing," signed by Dr. Carbone on August 5, 2014, and filed on August 7, 2014, in this matter (Ortwein v. CertainTeed Corporation, Alameda County Superior Court No. RG13701633). In paragraph 9 of that Declaration, Dr. Carbone declares as follows:

  - Individuals with the BAP1 mutation can develop mesothelioma without exposure to asbestos, as discussed in the Nature Review Cancer article and related references. In other words, the BAP1 mutation, [in] and of itself, is capable of causing mesothelioma and many other cancers. However, asbestos exposure may increase the risk of developing mesothelioma in carriers of BAP1 mutations.”

- Testa - Para 29 – “Inheritance of a BAP1 mutation (causing BAP1 Syndrome) renders the person unusually susceptible to carcinogenesis. 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: it does not cause the initiation, promotion, or development of cancer. However, those processes can be caused when a carcinogenic agent is introduced into the body, such as upon exposure to asbestos, because the body is less able to defend itself.”
Lung Cancers and Familial Mutations

• Lung cancer claiming has increased noticeably in asbestos litigation, and so experts and parties are starting to pay more attention to germline mutations for lung cancer claimants

• Increasing investigation of germline mutations by oncologists and others

• “DENVER – Two studies are providing new insight into germline epidermal growth factor receptor (EGFR) T790M mutation in familial non-small cell lung cancer (NSCLC). The findings suggest the need for tailored approaches for early detection and treatment, as well as for genetic testing to identify carriers


• “These studies now solidify the fact that routine clinical management of lung cancer now has to include the awareness of this inherited cancer syndrome," wrote David P. Carbone, MD, PhD, President-Elect of the International Association for the Study of Lung Cancer (IASLC), in an editorial.
Somatic Mutations and Possible “Signatures” for Lung Cancers After Inhalation of Asbestos
Asbestos “Signature” in Lung Cancer Tumors? – Nymark and Helsinki

- Nymark and other EU researchers are several years into looking at lung cancer tumors for molecular markers related to past asbestos exposure and perhaps causation
- The International Conference on Monitoring and Surveillance of Asbestos-Related Diseases on 11-13 February 2014 gathered together 140 experts from 23 countries – online at:
- Meeting produced a document referred to as a consensus document (some may not agree on the quality of the consensus) – available online at:
  - Asbestos, asbestososis, and cancer, the Helsinki criteria for diagnosis and attribution 2014: recommendations, Scand J Work Environ Health, doi:10.5271/sjweh.3462
- Paper identifies the following regarding possible molecular “signatures” in tumors for asbestos exposure and perhaps causation
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<td>Al and loss at 2p16</td>
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<td>Lung cancer of asbestos-exposed individuals</td>
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<td>Up-regulation of TP53</td>
<td>Decreased or abnormal tumor suppressor activity possibly due to mutations</td>
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<td>Serum Ras (p21)</td>
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<td>Specific mutations</td>
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Expanding Molecular Investigation of Tumors and Fibers
Recent Molecular Inputs Related to Mesothelioma Causation

- Recent animal study work compares the molecular events following inhalation of different types of asbestos fibers (crocidolite v. chrysotile).
- Finding indicate relatively consistent molecular level events, except the events are shorter term (perhaps 10 weeks of effects) for chrysotile fibers, which degrade much more quickly in vivo than do crocidolite or other amphibole fibers.
  - Fang Qi, Gordon Okimoto, Sandro Jube, Harvey I. Pass, Rozalia Laczko, Richard M. DeMay, Ghazal Khan, Maarit Tiirikainen, Haining Yang, Giovanni Gaudino, and Michele Carbone
  - Cancer Research: April 15, 2013; Volume 73, Issue 8, Supplement 1

- A very new paper (12/4/14) identifies polyclonal mesothelioma tumors, and the authors raise questions about how to think about tumors, metastases, and causation
- “Our data suggest that, in contrast to current dogma, recurrence may represent novel malignancies, occurring because of the carcinogenic “field effect” of asbestos, its related chronic inflammation, and/or because of ubiquitous genetic predisposition in patients carrying germline BAP1 mutations. Therefore, MM may arise from a large pool of independent and mostly covert cancers, as observed in some other malignancies. Accordingly, the multiple minuscule pleural nodules that are characteristically found on the pleura of early-stage MM patients are likely pre-malignant lesions or early tumors rather than early local metastases.
Other Molecular Impacts on Asbestos Litigation
Smoking – MicroRNA Fingerprints

- Asbestos defendants are becoming more interested in finding relatively inexpensive ways to implicate tobacco or other substances as causes of lung cancers, and perhaps even mesotheliomas
  - Some varying microRNA expression patterns found to date appear related to intake of heavy metals, synthetic estrogens, and tobacco smoke
- Schembri et al. compared lung tissues of smokers and non-smokers, and found that several microRNA were differentially expressed
    http://www.pnas.org/content/106/7/2319.short
- A 2012 paper by Russ and Slack provides a comprehensive overview of microRNA expression changes that appear to arise from smoking, and also discusses how those changes appear linked to the development of lung cancer.
    http://www.hindawi.com/journals/pm/2012/791234/
Other Molecular Developments and Asbestos Litigation

- Sensitive biomarkers emerging for mesotheliomas and malignant nodules

- Mesothelioma biobank in existence for mesotheliomas, with CDC support

- Some genetic testing information from commercial testing labs is appearing in medical records and then law suits because the person with cancer is a patient receiving “precision medicine” care, with sequencing for somatic mutations

- Increasing medical use of microRNA tests to determine if a tumor is a mesothelioma

- Increasing private lung cancer screening through low dose CT scans; CMS appears ready to approve Medicare payments as per tentative announcement in Nov. 2014
Genetic and Molecular Issues for Asbestos and Other “Toxic Tort” Litigation

• Conflicting expert opinions in BAP1 case illustrate some of the potential issues regarding how tort law will evaluate and define causation in a genetic and molecular age
  – Is intake of a “toxin” the legal cause of a cancer if the effective operation of some part of a person’s bodily system was previously reduced or blocked by a germline mutation?
  – What weight to give to past epidemiologic findings if a mutation (e.g. BAP1) creates a class of especially at risk persons for whom a lower dose of fibers may be “enough”
  – What standard of care applies to past and future manufacturing in an age of increasing knowledge of germline mutations and knowledge of molecular methods of actions
    • Failure to test? Failure to warn? Failure of design?

• At what point will juries be allowed to hear expert testimony regarding a purported “molecular fingerprint” of a past “exposure” and/or possible cause of a tumor
“For the last time, in trademark disputes, you don’t get to see any DNA evidence.”
Questions or follow-up?

• More slides with cites and links are available

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Background and Disclosures

• Since 1984, commercial and asbestos trial lawyer for large corporations involved in manufacturing (no work for insurers)
  – GAF (pipe insulation and other)
  – W.R. Grace (spray applied materials)
  – Pneumo Abex (friction products and other)
  – Various others – pumps, valves, machines
  – Many business cases, some related to costs of toxic torts and related insurance issues

• Principal in economic consulting firm (Gnarus) that advises on asbestos risks

• Pro bono director of not for profit focused on cancer issues, and pro bono legal work for persons with cancers when health insurers deny treatments

• GlobalTort blog on intersections of science and law – about page has more detail
GlobalTort Blog

- GlobalTort blog is located at [www.GlobalTort.com](http://www.GlobalTort.com)
- Focus is on intersections between science, law, and other disciplines
- Numerous articles on asbestos and other toxic tort litigation
- Updated 3-5 times per week, most weeks