Cancer Research Collaboration:
Michele Carbone, MD, PhD and Haining Yang, MD, PhD

Contact information

Michele Carbone, MD, PhD

Websites:

- www.oakparkpathology.com
- University of Hawaii Cancer Center

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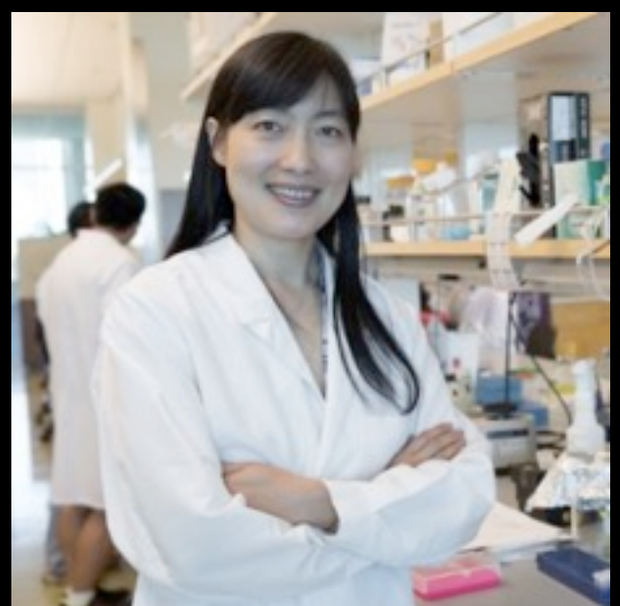


Haining Yang, MD, PhD

Website:

- University of Hawaii Cancer Center

Email - hyang@cc.hawaii.edu

“Save one life, and you have saved the world.”

- Our central theme: Gene and Environment Interaction in Mesothelioma
  - What causes mesothelioma to develop in some people and not others?
  - Can we prevent mesothelioma?
  - Can we cure mesothelioma?
  - Can we improve the lives of people who get mesothelioma?

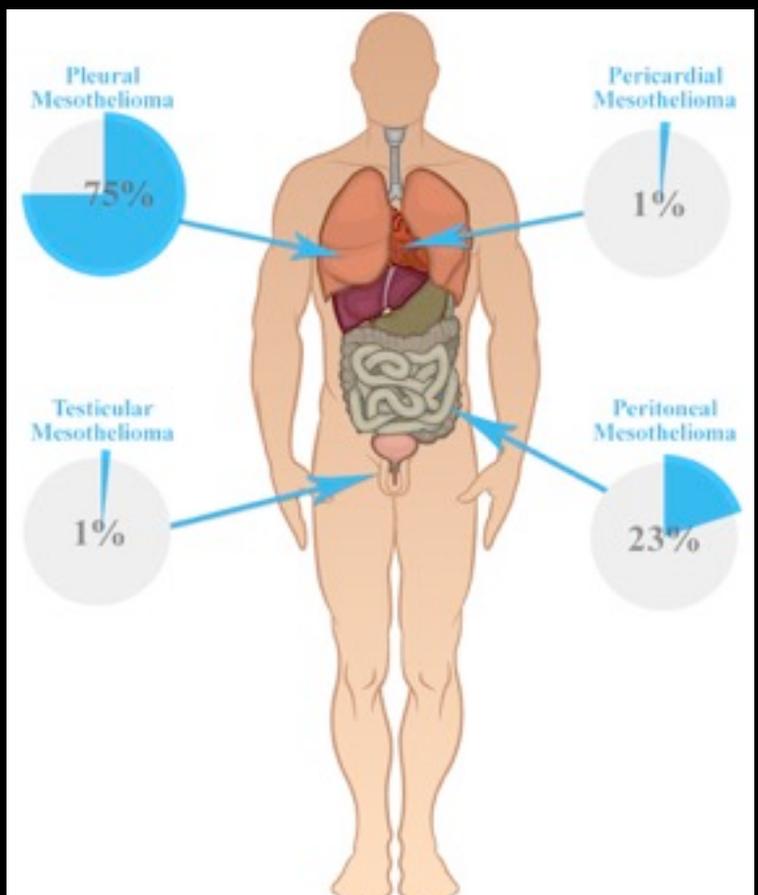
Our Research Projects

BAP1 – Genetic Alterations

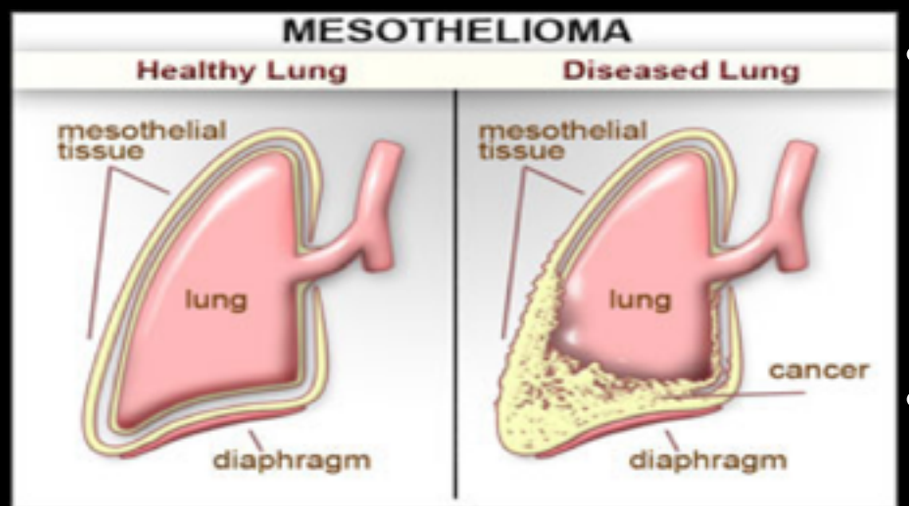
Environmental

Asbestos Carcinogenesis & HMGB1

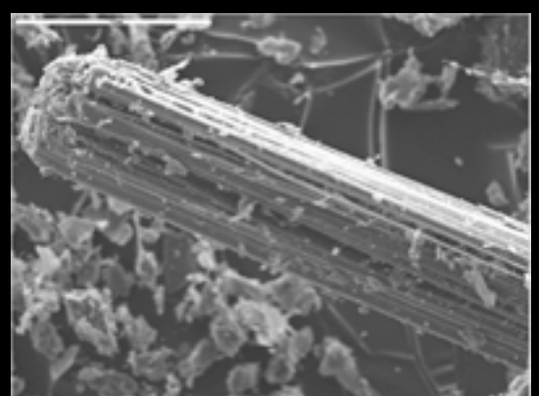
Mesothelioma Background Information



Introduction to Mesothelioma

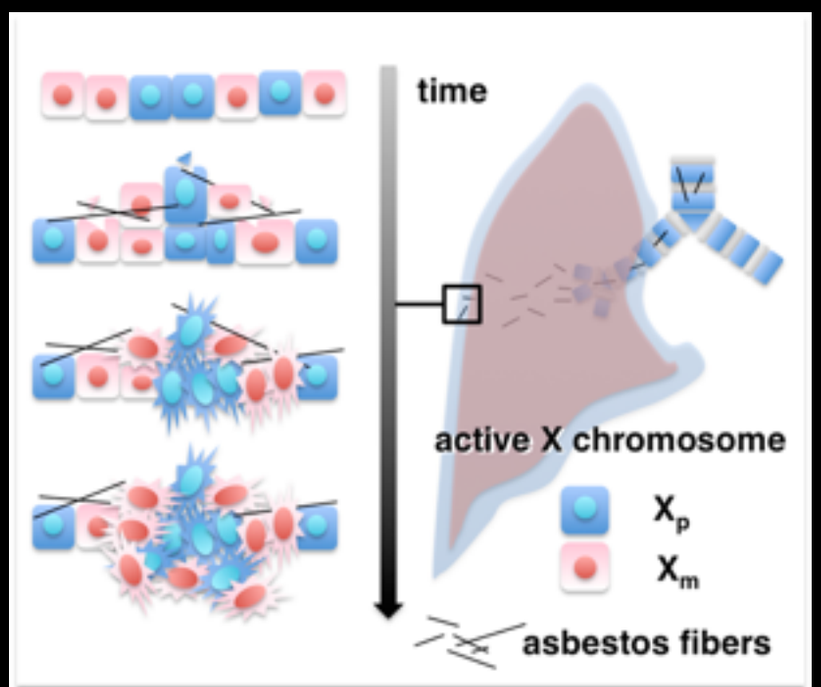


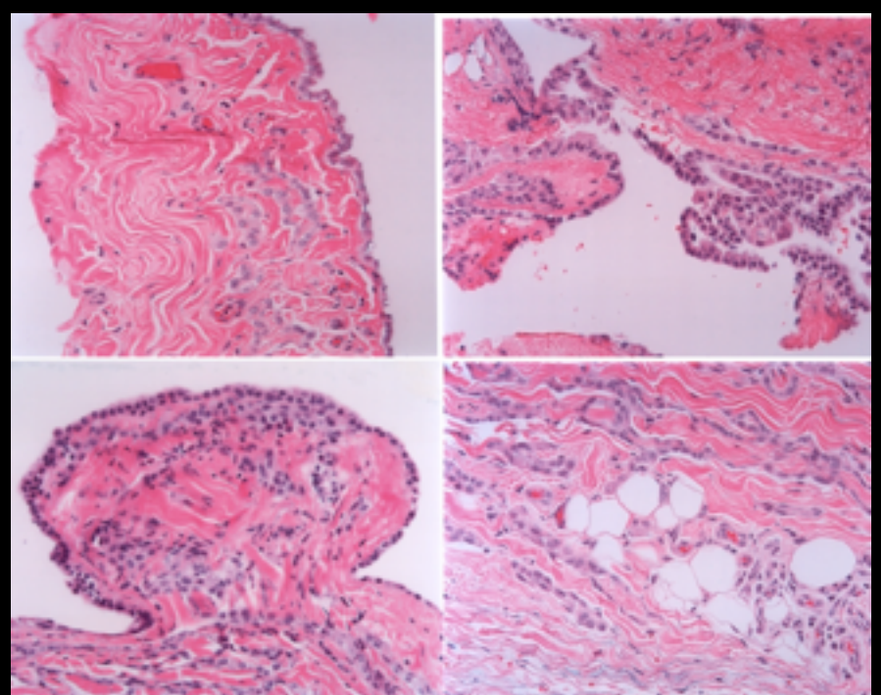
- Malignant mesothelioma (MM) is almost always fatal
  - ~3,000 US deaths per year
  - ~5,000 European deaths per year



- Asbestos and erionite exposure cause most cases of MM
- Latency period between initial asbestos exposure and MM is between 30 – 60 years
- Roughly 4.6% of individuals who worked in cocidolite asbestos minutes for 10+ years developed MM

Malignant mesotheliomas are polyclonal tumors

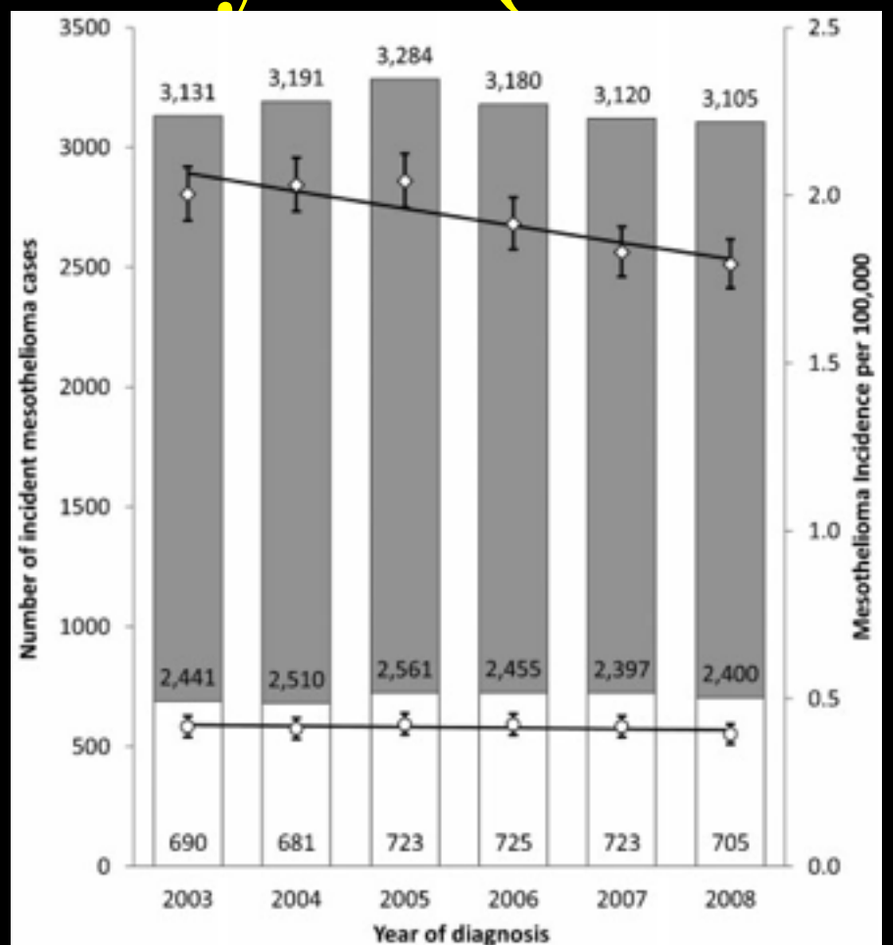




This creates an obvious problem with immunotherapy

Journal of Translational Medicine, Sep 2014, Evaluation of Clonal Origin of Malignant Mesothelioma, Comertpay,...Yang H, Carbone M.

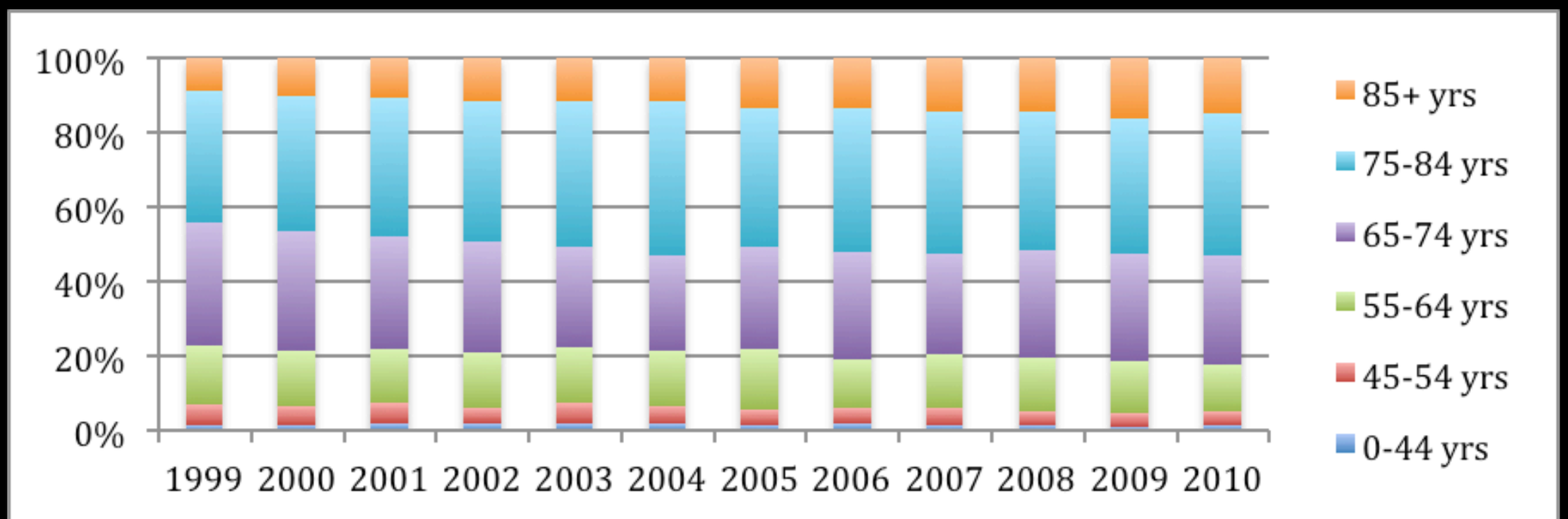
MM cases diagnosed in the US by sex and year (2003–2008)



Relatively Stable US Incidence

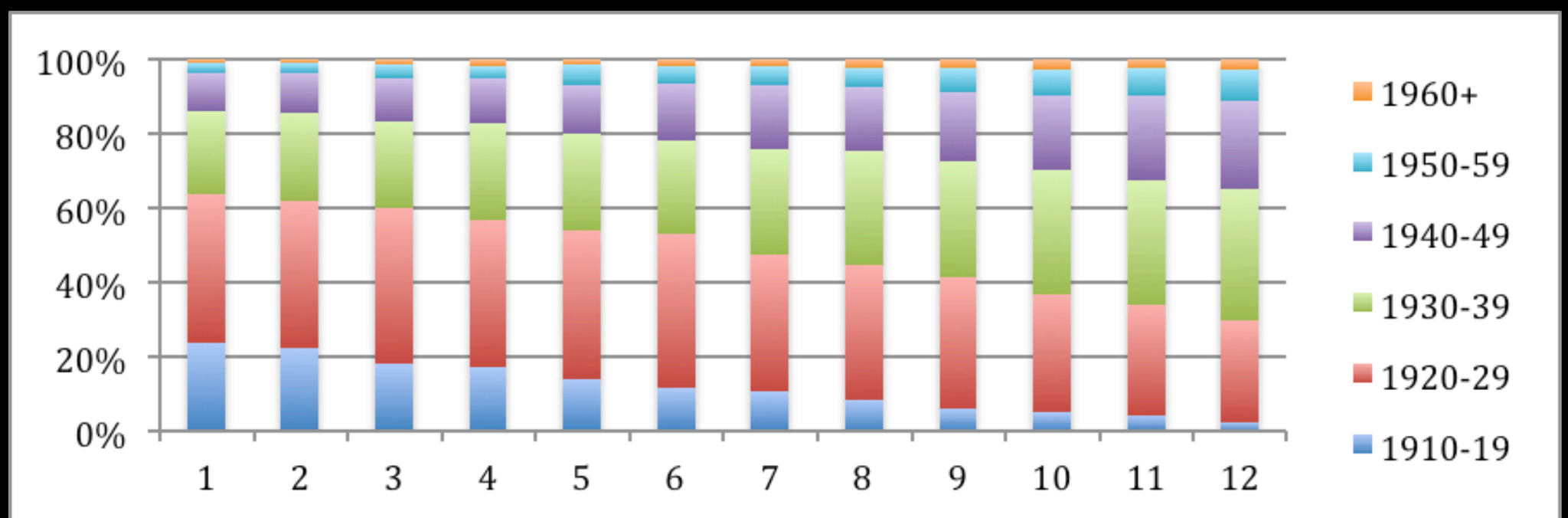
- Males have a stable incidence of mesothelioma since 2003
- Mesothelioma incidence in women has basically been unchanged for decades
  - Women are rarely professionally exposed to asbestos

Distribution of MM by age group per year (US: 1999-2010)



Baumann and Carbone, J Toxicol Environ Health, Part B, 19:231-249, **2016**

Proportion of MM cohorts per year in the US: 1999-2010



Baumann and Carbone, J Toxicol Environ Health, Part B, 19:231-249, **2016**

Research Funding: 12/2014 – 12/2016

- Grants to Michele Carbone (MC), Haining Yang (HY) and Harvey Pass (HIP):
  - P30 CA071789 to MC
  - V-Foundation 2013-16 to MC & HY
  - 1R01CA198138-01 2015-20 to MC
  - 1R01CA160715-0A 2011-17 to HY
  - DoD Team Translational grant 2016-2019 to HY and MC
  - DoD Idea grant 2017-18 To MC and HY
  - NCI-EDRN U01 2017-2022 to HY and HIP
  - UH Foundation through unrestricted donations from Honeywell International and from UNITED-FOR-A-CURE to MC Open
- MC and HY have 4 pending patents on BAP1
- MC and HY have been awarded 1 patent on BAP1 and HMGB1

Ongoing Collaborations

- HI. Pass, NYU, New York, US
- Wei Jia and Ian Pagano Honolulu, US
- M. Emi and Y. Yoshikawa, Yokohama, Japan
- W. Mao, Hangzhou, China
- Max Coppes and Joe Grzymski , U. Nevada and Desert Research Institute, Reno, US
- M. Bocchetta Loyola Chicago, US
- E. Taioli, M. Sinai, New York, US
- P. Pinton, Ferrara, Italy
- M. Russo, A. Napolitano, L. Pellegrini, Roma, Italy
- T. Mak, Toronto, Canada
- S. Kanodia, Cedar Sinai Los Angeles, US
- G.Gaudino, Isabelle Opitz, U. Zurich, Switzerland
- S. Emri, M. Metintash, Istanbul, Turkey

Progress Review 12/2014 – 12/2016

- 32 peer-reviewed publications
- 20 original research papers
  - We have been the First or Senior authors on 15 of 20 papers
- 6 review articles
  - We have been First or Senior authors in 5 out of 6 reviews
- 2 Scientific correspondence publications
  - We have been Senior author on each of those correspondences
- 4 Chapters in books
  - We have been First or Senior authors on 2 of the 4 chapters

Peer Reviewed Publications (12/2014 – 12/2016)

1. Comertpay S, Pastorino S, Tanji M, Mezzapelle R, Strianese O, Napolitano A, Baumann F, Weigel T, Friedberd J, Sugarbaker P, Kruasz T, Wang E, Powers A, Gaudino G, Kanodia S, Pass H, Parsons B, **Yang H**, **Carbone M**. Evaluation of clonal origin of malignant mesothelioma. *J Transl Med*, 12: 301-306, **2014**.
2. Baumann F, Flores E, Napolitano A, Kanodia A, Taioli E, Pass H, **Yang H**, **Carbone M.** Mesothelioma Patients with Germline BAP1 Mutations Have Seven-Fold Improved Long-term Survival. *Carcinogenesis*, 36:76-81, **2015**.
3. Guo G, Chmielecki J, Goparaju C, Heguy A, Dolgalev I, **Carbone M**, Seepo S, Meyerson M, Pass HI. Whole exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A and CUL1 in malignant pleural mesothelioma. *Cancer Res,*75:264-9, **2015**.
4. Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Baumann F, Zhang YA, Gazdar A, Kanodia S, Tiirikainen M, Flores E, Gaudino G, Becich GJ, Pass HI, **Yang H**, **Carbone M**. High incidence of somatic BAP1 alterations in sporadic malignant mesothelioma. *J Thorac Oncol*, 10:565-76, **2015**.
5. Baumann F, Buck BJ, Metcalf RV, McLaurin BT, Merkler D, **Carbone M**. The presence of asbestos in the natural environment is likely related to mesothelioma in young individuals and women from Southern Nevada. *J Thorac Oncol*, 10:731-7, **2015**.

Peer Reviewed Publications (12/2014 – 12/2016, continued)

6. Hoffmann PR, Panigada M, Soprana E, Terry F, Bandar IS, Napolitano A, Rose AH, Hoffmann FW, Ndhlovu LC, Belcaid M, Moise L, De Groot AS, **Carbone M**, Gaudino G, Matsui T, Siccardi A, Bertino P. Preclinical development of HIvax: Human survival in highly immunogenic vaccines. *Hum Vaccin Immunother*, 11:1585-95, **2015**.

7. Croce A, Allegrina M, Rinaudo C, Gaudino G, **Yang H**, **Carbone M**. Numerous iron-rich particles lie on the surface of erionite fibers from Rome (Oregon, USA) and Karlik (Cappadocia, Turkey). *Microsc Microanal*, 21:1341-7, **2015**.

8. Daou S, Hammond-Martel I, Mashtalir N, Barbour H, Gagnon J, Iannantuono NV, Sen Nkwe N, Motorina A, Pak H, Yu H, Wurtele H, Milot E, Mallette FA, **Carbone M**, Affar EB. The BAP1/ASXL2 Histone H2A Deubiquitinase Complex Regulates Cell Proliferation and is Disrupted in Cancer. *J Biol Chem*, 290: 28643–63, **2015**.

9. **Yang H**, Pellegrini L, Napolitano A, Giorgi C, Jube S, Preti A, Jennings CJ, De Marchis F, Flores EG, Larson D, Pagano I, Tanji M, Powers A, Kanodia S, Gaudino G, Pastorino S, Pass HI, Pinton P, Bianchi ME, **Carbone M**. Aspirin delays mesothelioma growth by inhibiting HMGB1-mediated tumor progression. *Cell Death Dis,* 6:e1786, **2015.**

10. **Carbone M,** Flores EG, Emi M, Johnson TA, Tsunoda T, Behner D, Hoffman H, Hesdorffer M, Nasu M, Napolitano A, Power A, Minaai M, Baumann F, Bryant-Greenwood P, Lauk O, Kirschner MB, Weder W, Opitz I, Pass HI, Gaudino G, Pastorino S, **Yang H**. Combined genetic and genealogic studies uncover a large BAP1 cancer syndrome kindred, tracing back nine generations to a common ancestor from the 1700s. *PLOS Genet*, 11(12): e1005633, **2015**.

Peer Reviewed Publications (12/2014 – 12/2016, continued)

11. Napolitano, A, Pellegrini L, Dey A, Larson D, Tanji M, Flores EG, Kendrick B, Lapid D, Powers A, Kanodia S, Pastorino S, Pass HI, Dixit V, **Yang H**, **Carbone M**. Minimal asbestos exposure in germline *BAP1* heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. *Oncogene*, 35:1996-2002, **2016**.

12. Pass HI, Goparaju C, Espin-Garcia O, Donington J, **Carbone M**, Patel D, Chen Z, Feld R, Cho J, Gadgeel S, Wozniak A, Chachoua A, Leighl N, Tsao MS, de Perrot M, Xu W, Liu G. Plasma Biomarker Enrichment of Clinical Prognostic Indices in Malignant Pleural Mesothelioma. *J Thorac Oncol*, 11:900-9, **2016**.

13. Napolitano A, Antoine DJ , Pellegrini L, Baumann F, Pagano IS , Pastorino S, Goparaju CM, Prokrym K, Canino C, Pass HI, **Carbone M, Yang H**. HMGB1 and its hyper-acetylated isoform are sensitive and specific serum biomarkers to detect asbestos exposure and to identify mesothelioma patients. *Clin Cancer Res*, 22:3087-96, **2016**.

14. 14. Zhang L, Shimizu D, Killeen JL, Honda SA, Lu D, Stanoyevitch A, Lin F, Wang B, Monuki ES**, Carbone M**. Serous Carcinoma component championed by Heparin Binding-EGF Like Growth Factor (HB-EGF) Predisposing to Metastasis and Recurrence in Stage I Uterine Malignant Mixed Mullerian Tumor. *Hum Pathol*, 53:159-67, **2016**.

15. **Carbone M**, Kanodia S, Chao A, Miller A, Wali A, Weissman D, Adjei A, Baumann F, Boffetta P, Buck B, de Perrot M, Dogan AU, Gavett S, Gualtieri A, Hassan R, Hesdorffer M, Hirsch FR, Larson D, Mao W, Masten S, Pass HI, Peto J, Pira E, Steele I, Tsao A, Woodard GA, **Yang H**, Malik S. Consensus Report of the 2015 Weinman International Conference on Mesothelioma. *J Thorac Oncol*, 11:1246-1262, **2016**.

Peer Reviewed Publications (12/2014 – 12/2016, continued)

16. **Carbone M**, Shimizu D, Napolitano A, Tanji M, Pass HI, **Yang H**, Pastorino S. Positive nuclear BAP1 immunostaining helps differentiate non-small cell lung carcinomas from malignant mesothelioma. *Oncotarget*, **2016** Jul 18. doi: 10.18632/oncotarget.10653, advance online publication.
17. Larson D, Powers A, Ambrosi JP, Tanji M, Napolitano A, Flores EG, Baumann F, Pellegrini L, Jennings CJ, Buck BJ, McLaurin BT, Merkler D, Robinson C, Morris P, Dogan M, Dogan AU, Pass HI, Pastorino S, **Carbone M**, **Yang H**. Investigating palygorskite's role in the development of mesothelioma in southern Nevada: Insights into fiber-induced carcinogenicity. *J Toxicol Environ Health, Part B*, 19:213-230, **2016**.
18. Yoshikawa Y, Emi M, Hashimoto-Tamahoki T, Ohmuraya M, Sato A, Tsujimura T, Hasegawa S, Nakano T, Nasu M, Pastorino S, Szymiczek A, Bononi A, Tanji M, Pagano I, Gaudino G, Napolitano A, Goparajiu C, Pass HI, **Yang H**, **Carbone M**. High-density array-CGH with targeted NGS unmask multiple non-contiguous minute deletions on chromosome 3p21 in mesothelioma. *Proc Natl Aca Sci USA*, In Press, Nov. 2016
19. Mao W, Zhang X, Guo Z, Gao Z, Pass HI, **Yang H**, **Carbone M.** Mesothelioma in Eastern China is Mostly Prevalent Among Young Women. *JAMA Oncol*, In press Dec 1, 2016
20. Guo Z, **Carbone M**, Zhang X, Su D, Sun W, Lou J, Gao Z, Shao D, Chen J, Zhang G, Hu J, Chen K, Wang F, Pass HI, Yu H, **Yang H**, Mao W. Improving the accuracy for mesothelioma diagnosis in China. *J Thorac Oncol* ***Dec 2016***

6 Reviews (12/2014 – 12/2016)

1. **Carbone M**, Gaudino G, **Yang H**. Recent insights emerging from malignant mesothelioma genome sequencing. *J Thorac Oncol*, 10:409-11, **2015**.
2. Klebe S, Driml J, Nasu M, Pastorino S, Zangiabadi A, Henderson D, **Carbone M**. BAP1 hereditary cancer predisposition syndrome: a case report and review of literature. *Biomark Res*, 3:14, **2015**.
3. Bononi A, Napolitano A, Pass HI, **Yang H**, **Carbone M**. Latest developments in our understanding of the pathogenesis of mesothelioma and the design of targeted therapies. *Expert Rev Respir Med*, 9:633-54, **2015**.
4. Ransohoff KJ, Jaju PD, Tang JY, **Carbone M**, Leachman S, Sarin KY. Familial skin cancer syndromes: Increased melanoma risk. *J Am Acad Dermatol*, 74:423-34, **2016**.
5. Baumann F, **Carbone M**. Environmental risk of mesothelioma in the U.S.: An emerging concern - epidemiological issues. *J Toxicol Environ Health, Part B*, 19:231-249, **2016**.
6. Napolitano A, **Carbone M**. Malignant mesothelioma: Time to translate? *Trends In Cancer*, 2:467-74, **2016**.

2 Scientific Correspondences(12/2014 – 12/2016)

1. Baumann F, Buck BJ, Metcalf RV, McLaurin BT, Merkler D, **Carbone M**. Reply to "No increased risk for mesothelioma in relation to natural-occurring asbestos in Southern Nevada". *J Thorac Oncol*, 10:e64-65, **2015**.
2. Napolitano A, **Carbone M**. Letter to Editor "Concerns about presence of a wild-type BAP1 allele in absence of nuclear protein expression". *JAMA Dermatol*, 151:1265-1266, **2015**.

4 Book Chapters (12/2014– 12/2016)

1. Napolitano A, Jube S, Gaudino G, Pass HI, **Carbone M**, **Yang H**. Asbestos-induced chronic inflammation and cancer. *Cancer and Inflammation Mechanisms: Chemical, Biological, and Clinical Aspects*, Hiraku Y, Kawanishi S, Ohsima H (eds.). John Wiley & Sons, Inc.: Hoboken, New Jersey, pp. 223-234, **2014**.
2. Pass HI, **Carbone M**, Krug LM, Rosenzweig K. Benign and Malignant Mesothelioma. In: *Cancer: Principles and Practice of Oncology*, 10th ed. De Vita VT, Lawrence TS, and Rosenberg SA. (Eds). Baltimore: Lippincott, Williams and Wilkins; **2014**.
3. Napolitano A, Pellegrini L, **Yang H**, **Carbone M**. Somatic and germline BAP1 mutations in malignant mesothelioma. In: Malignant Pleural Mesothelioma: Present Status and Future Directions, 1st ed. Mineo TC (Ed). Sharjah U.A.E: Bentham Science Publishers Ltd.; **2016.** ISBN: 978-1-68108-193-9.
4. **Carbone M**, **Yang H**. Biological Activities of Asbestos and Other Mineral Fibers. In: Gualtieri, AF (Ed), *European Mineralogical Union, EMU Notes in Mineralogy*, *Vol 17*; *Mineral fibres: crystal chemistry, chemical-physical properties, biological interaction and toxicity.* European Mineralogical Union and the Mineralogical Society of Great Britain and Ireland.**, In press**.

Research Project 1: BAP1

BAP1 Project Findings

- **The most powerful gene that regulates Gene X Environment interaction in human cancer**
- **BAP1 was discovered in 1997: 14 total publications from 1997-2011**
- **In 2011 we discovered that germline BAP1 mutations cause a novel cancer syndrome that we named "BAP1 cancer syndrome" with high frequency of mesothelioma, eye melanoma, etc.,**
  - **<u>and</u> that BAP1 acquired somatic mutations are frequent in sporadic (non-hereditary) mesothelioma, underscoring the key role of BAP1 in this cancer.**
- **Since we published our paper in 2011 there have been over 450 publications on BAP1, 417 of which cited our 2011 paper (6/1/17)**

Gene X Environment interaction in mesothelioma (How it started)

In 1997 I (Carbone) started working in 3 villages in Cappadocia where there is an epidemic of MM

Roushdy-Hammady and Carbone M., The Lancet 2001; Carbone M et al. Nat. Rev

My "office" in Sarhidir, next to me Drs. Baris, opposite Dr. Emri with whom I conducted this research

Why some families had much more mesothelioma than others? Could be genetics?

Father died of malignant mesothelioma

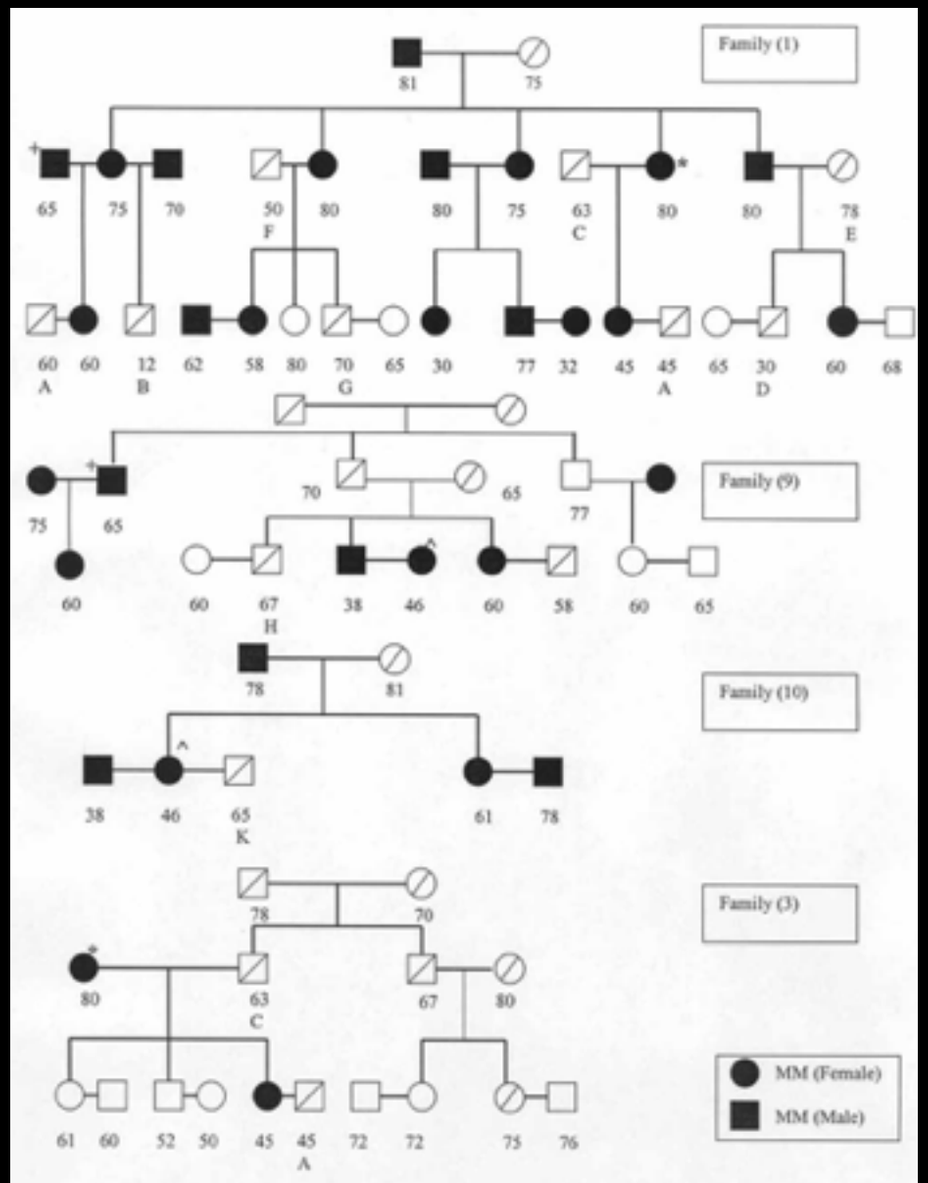
Mother died of malignant mesothelioma

Cappadocia

We discovered that genetics predispose some people exposed to mineral fibers to mesothelioma.

Black dots represent family members who died of mesothelioma.

At that time many people thought we were crazy to propose that genetics influences mineral fiber carcinogenesis. Today everybody accepts that.

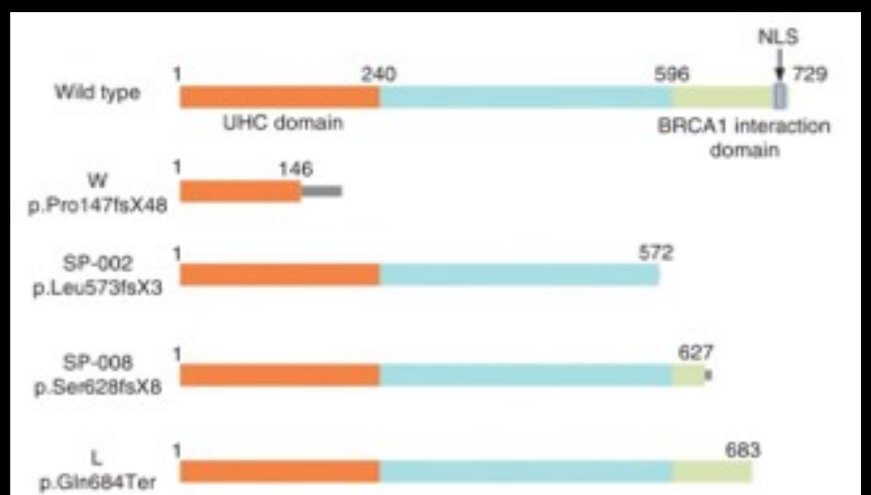


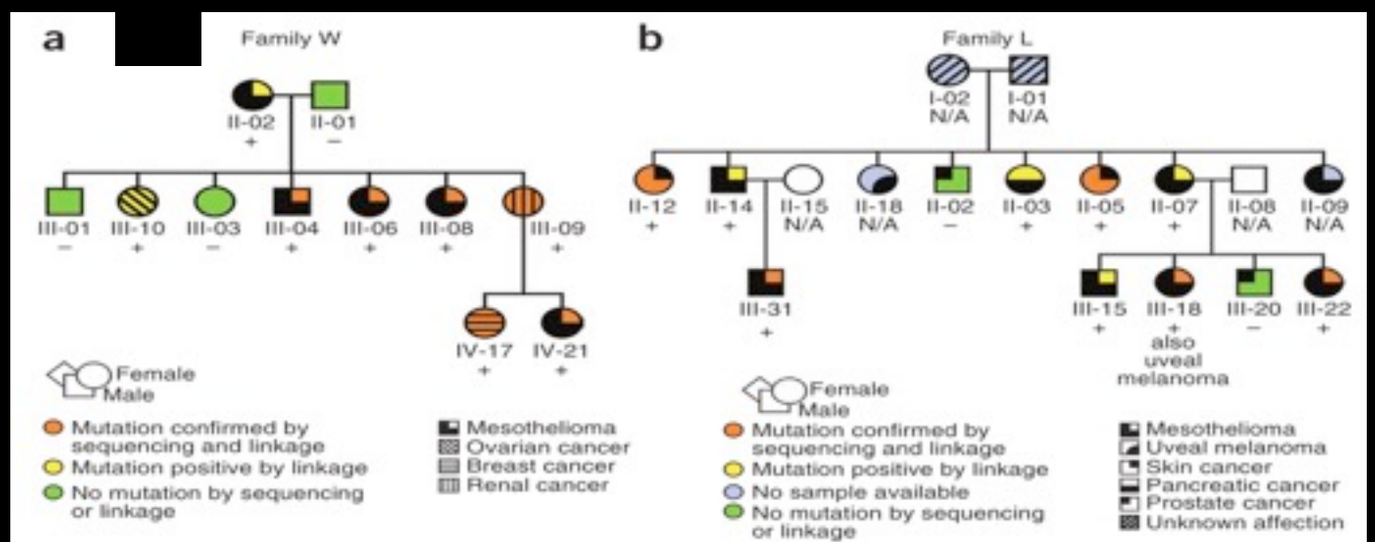
The new village of Tuzkoy to reduce exposure Carbone M, et al Nature Reviews Cancer, February 2007

Dr. Carbone with Dr. Baris in the newly built erionite-free Tuzkoy village in Cappadocia. The village was built to address the environmental problems we uncovered. In 2015 also the village of Karain was rebuilt with erionite free material to prevent MM in future generations.

Top, Tuzkoy's Mesothelioma Clinic, built in response to my request to then Director of Cancer Control, Dr. Murat Tuncer. Below official opening, 2009. Officers of Turkish Ministry of Health, villagers, Drs. Carbone, Pass, Yang.

We studied 2 US families with very high incidence of malignant mesothelioma





- Our research proved the role of genetics in causing malignant mesothelioma
- We named this condition the “BAP1 syndrome”

We studied 2 US families with very high incidence of malignant mesothelioma

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]

VOLUME 43 | NUMBER 10 | OCTOBER 2011 NATURE GENETICS

nature REVIEWS CANCER

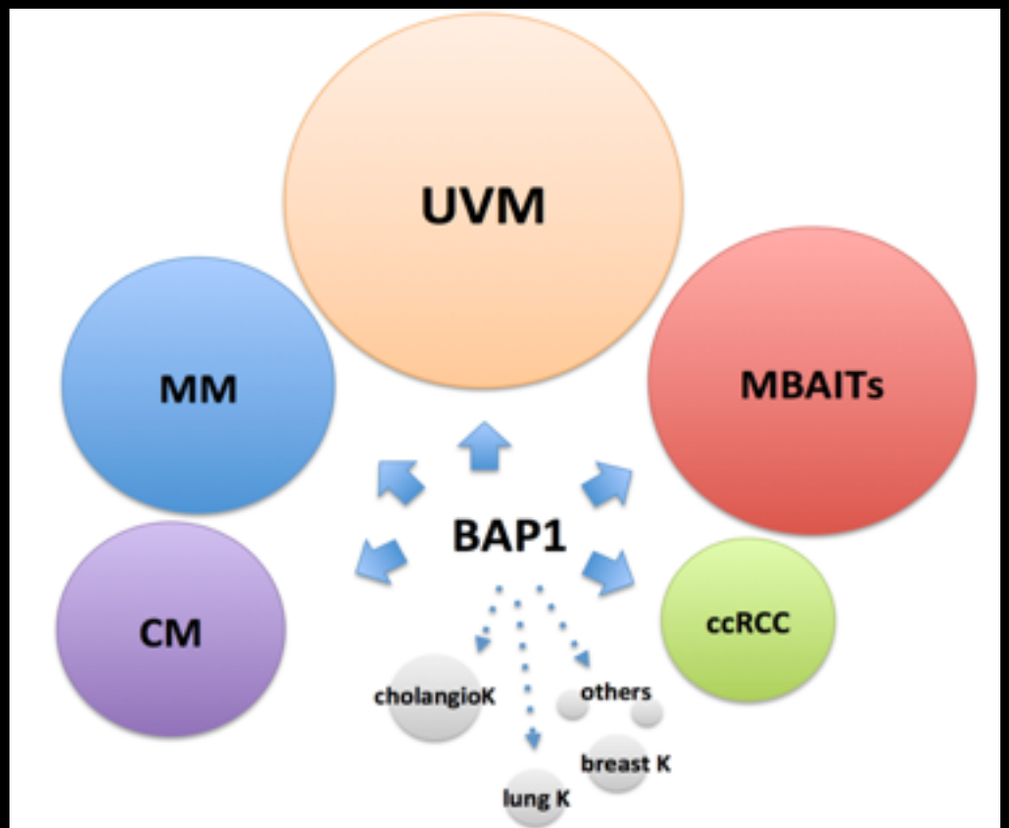
Progress

Nature Reviews Cancer **13**, 153-159 (March 2013) | doi:10.1038/nrc3459

BAP1 and cancer

Michele Carbone, Haining Yang, Harvey I. Pass, Thomas Krausz, Joseph R. Testa & Giovanni Gaudino

The BAP1 Cancer Syndrome



Carriers of BAP1 mutations are predisposed to different tumors:

- Malignant mesothelioma
- Uveal and cutaneous melanoma
- Renal Cell carcinoma, clear cell type
- Basal cell and squamous carcinomas
- Cholangioca and other cancers
- MBAITS (benign melanocytic tumors)

- Nature Reviews Cancer, Progess: BAP1 and Cancer, March 2013, Carbone M, et al.
- Journal of Translational Medicine, BAP1 cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MBAITS, August 2012, Carbone M, ..., Harvey I Pass and Yang H

Findings in mice

- We recently discovered that mice with germline BAP1 mutations are more susceptible to asbestos carcinogenesis

Oncogene (2015), 1–7

www.nature.com/onc

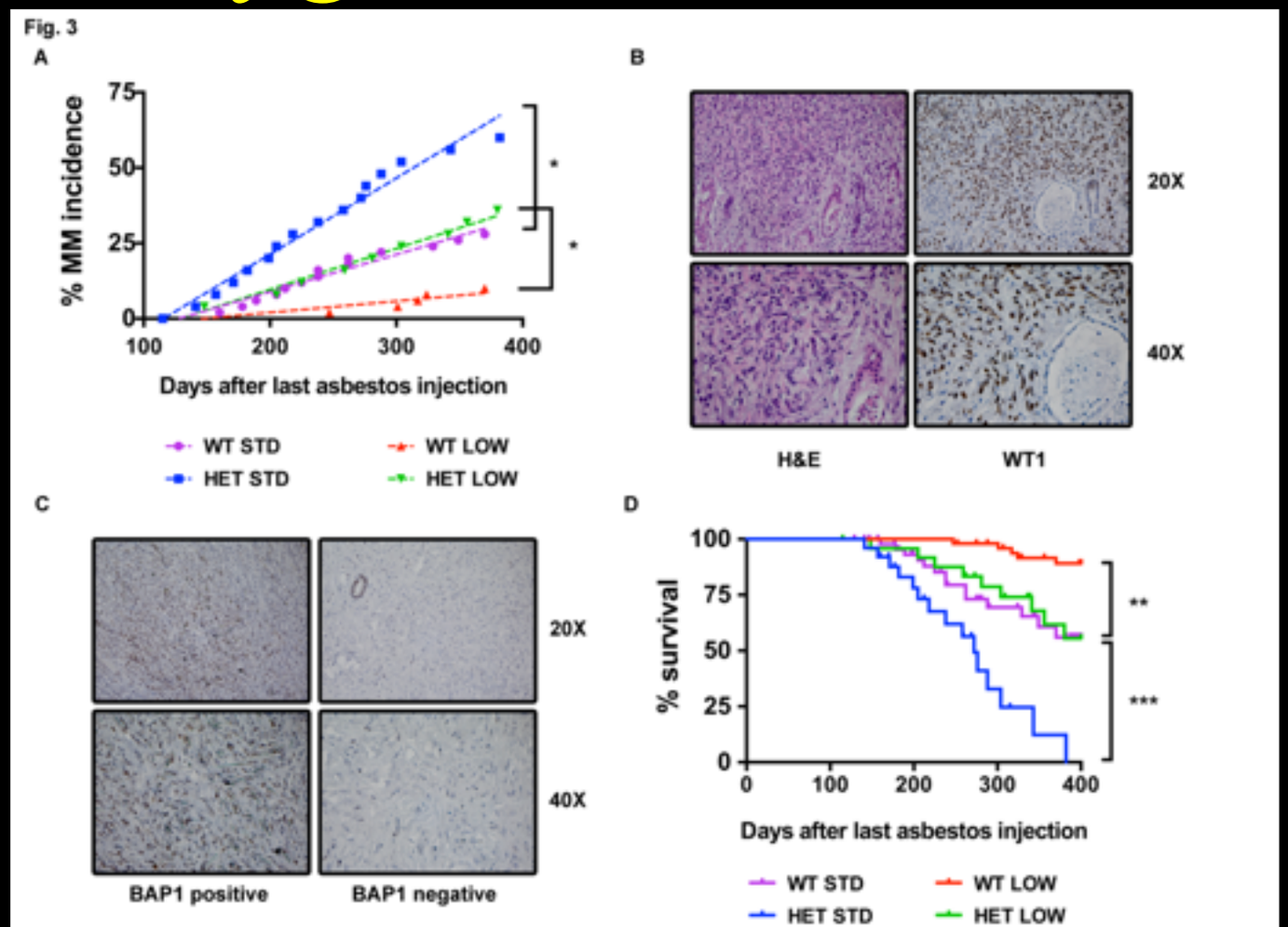
SHORT COMMUNICATION

Minimal asbestos exposure in germline *BAP1* heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma

A Napolitano[1,2], L Pellegrini[1], A Dey[3], D Larson[1], M Tanji[1], EG Flores[1], B Kendrick[1], D Lapid[1], A Powers[1], S Kanodia[4], S Pastorino[1], HI Pass[5], V Dixit[3], H Yang[1] and M Carbone[1]

Mice with heterozygous BAP1…

- We mimic the human condition of those affected with the BAP1 cancer syndrome
- The mice with these mutations have a much higher incidence of mesothelioma when exposed to even small amounts of asbestos



Tracing BAP1 in some US families

- We traced the origin of BAP1 mutations in some US families
- We found the original couple, who immigrated from Europe in 1717
- We published our results in PLOS Genetics in December 2015

PLOS | GENETICS

RESEARCH ARTICLE

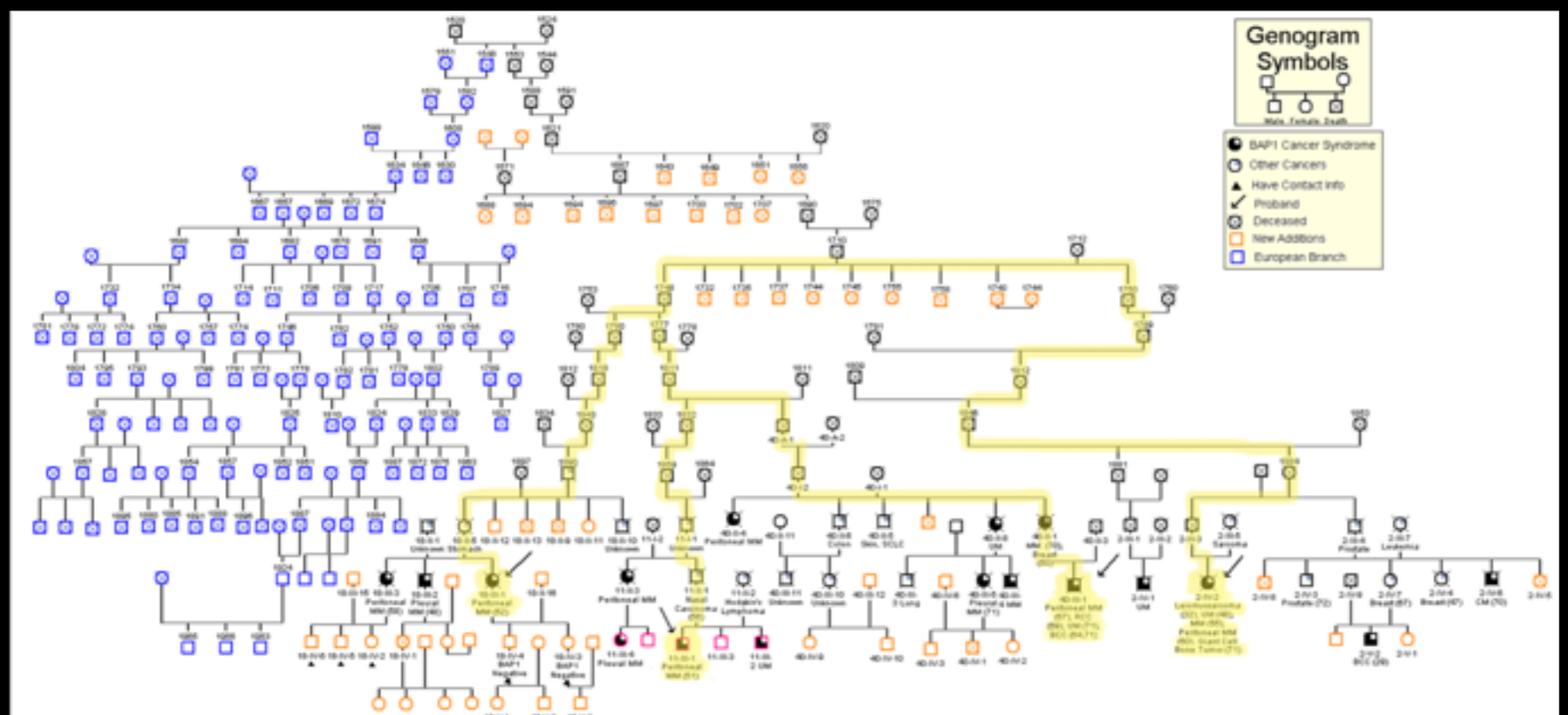
Combined Genetic and Genealogic Studies Uncover a Large BAP1 Cancer Syndrome Kindred Tracing Back Nine Generations to a Common Ancestor from the 1700s

Michele Carbone[1&*], Erin G. Flores[1&], Mitsuru Emi[1&], Todd A. Johnson[2], Tatsuhiko Tsunoda[2], Dusty Behner[1], Harriet Hoffman[3], Mary Hesdorffer[4], Masaki Nasu[1], Andrea Napolitano[1], Amy Powers[1], Michael Minaai[1], Francine Baumann[1], Peter Bryant-Greenwood[1], Olivia Lauk[5], Michaela B. Kirschner[5], Walter Weder[5], Isabelle Opitz[5], Harvey I. Pass[6], Giovanni Gaudino[1], Sandra Pastorino[1], Haining Yang[1*]

Some of the descendants presently live in Texas and California

Carbone et al., PLOS Genetics December, 2015

Links to anscestors living in Switzerland in the 1400s



Carbone et al., PLOS Genetics December, 2015

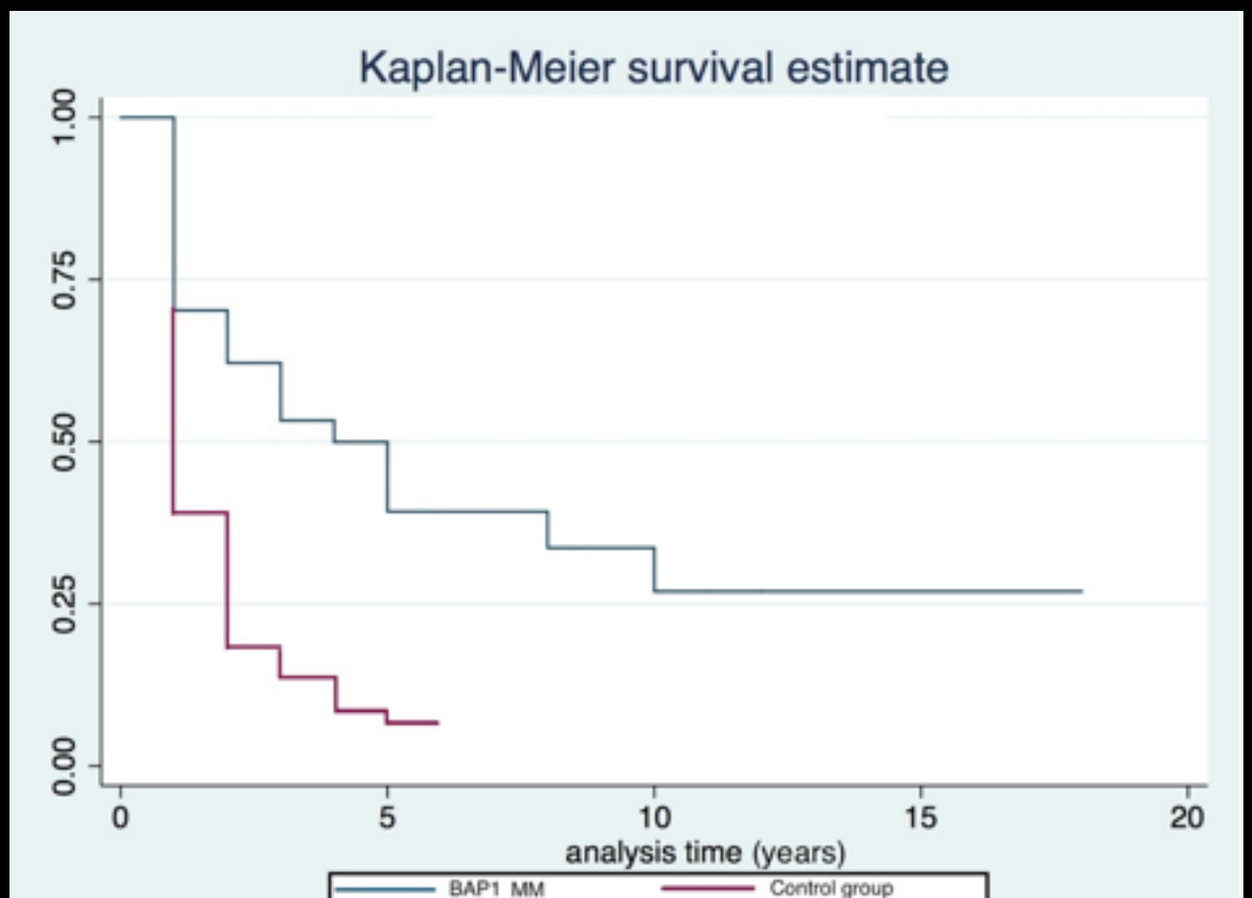
Implications of germline BAP1 mutations

- Individuals with BAP1 germline mutations develop cancer
- However, they have less aggressive tumors compared to the same tumor types when they develop sporadically
- For example, our publication Germline BAP1 Mutations and Survival was published in Biomarker Research (3:14-21, 2015)
  - 72 year old woman with no history of asbestos exposure
  - 5+ years survival after being diagnosed with
    - Uveal melanoma
    - Peritoneal malignant mesothelioma
    - Intrahepatic cholandiocarcinoma
- Germline BAP1 mutation exon 7, catalytic domain predicted to impair BAP1 deubiquitinating activity

MM patients with germline BAP1 mutations have 7-fold improved long-term survival

Carcinogenesis 2015, Francine Baumann, Erin Flores, Andrea Napolitano, Shreya Kanodia, Emanuela Taioli, Harvey Pass, Haining Yang, and Michele Carbone

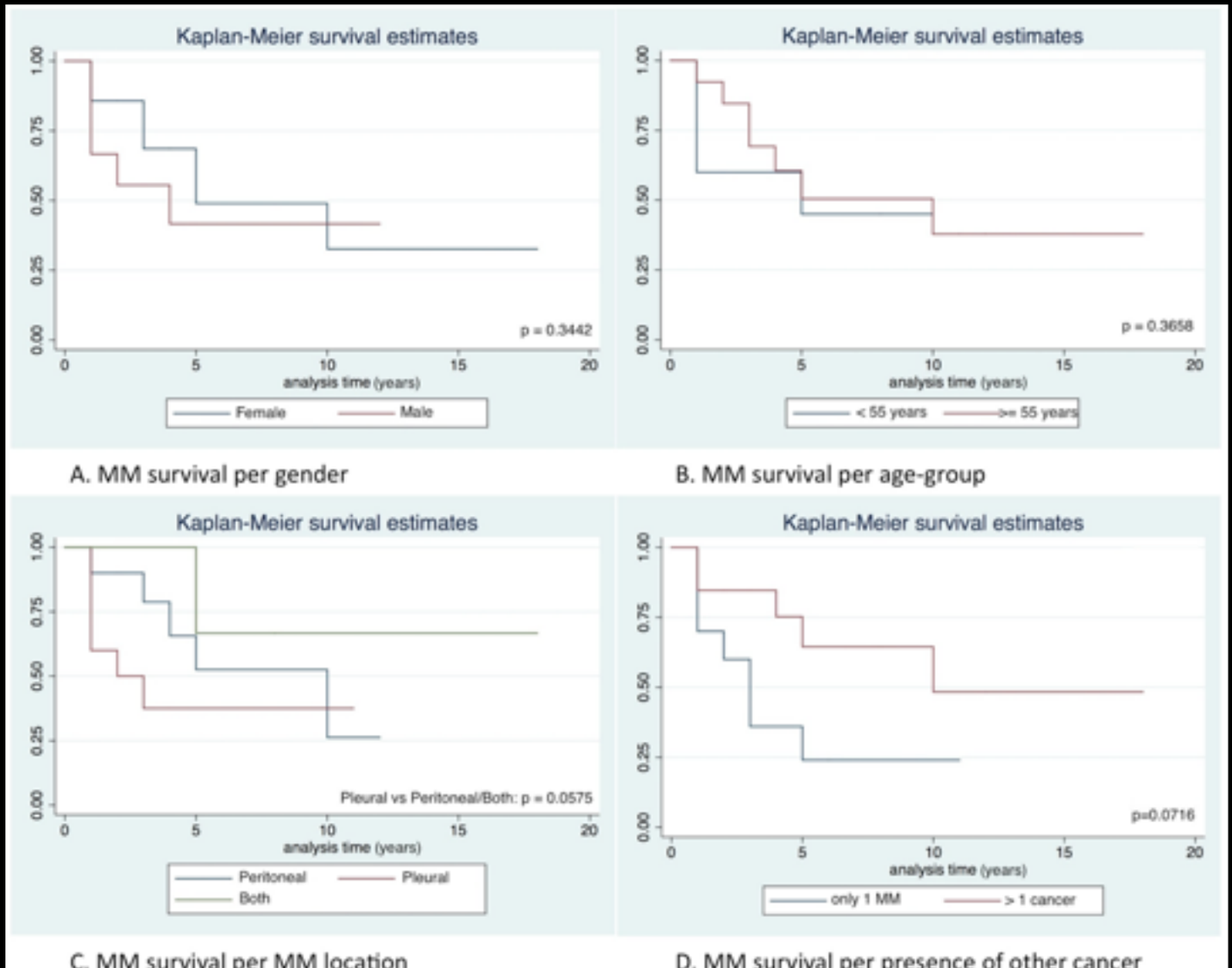
Survival comparisons



KEY

--- Polulation at large

--- Population with BAP1 germline mutations



KEY

--- Polulation at large

--- Population with BAP1 germline mutations

BAP1 negative staining helps pathologist diagnose mesothelioma

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2016

Positive nuclear BAP1 immunostaining helps differentiate non-small cell lung carcinomas from malignant mesothelioma

Michele Carbone[1], David Shimizu[2], Andrea Napolitano[1], Mika Tanji[1], Harvey I. Pass[3], Haining Yang[1], Sandra Pastorino[1]

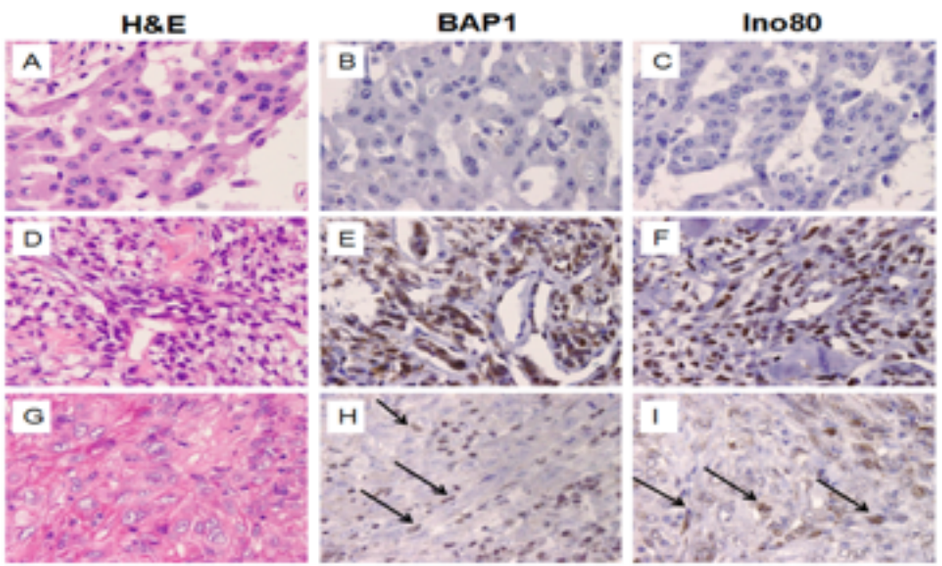


Table 3 BAP1 staining

	n	BAP1	Calretinin	WT-1	D2-40	CAM5.2
Total Lung Cancers	20	30 (100%)	6 (20%)	0 (0%)	24 (40%)	20 (100%)
Adenocarcinoma	10	10(100%)*	2(20%)**	0	4(40%)	10(100%)
Squamous cell carcinoma	10	10(100%)	4(40%)**	0	8(80%)	10(100%)
Carcinosarcoma of (all)	15	15(100)%	4(40%)**	0***	12(80%)	13(87%)#
Lung	5	5	1	0	4	5
Esophagus	1	1	0	0	1	0
Mediastinum	1	1	0	0	1	1
Breast	1	1	0	0	1	1
Pancreas	1	1	1	0	0	0
Cervix	2	2	1	0	2	1
Liver	1	1	1	0	0	1
Stomach	1	1	0	0	1	1
Duodenum	1	1	0	0	1	1
Total Mesotheliomas	22	8 (36.3%)	16	19	21	21
Epithelial	17	3 (17.6%)	14	16	16	17
Biphasic	3	2 (67%)	2	3	3	3
Sarcomatoid	2	2 (100%)	0	0	2	1

* All tumors showed homogeneous BAP1 nuclear staining in 100% of tumor cells, except for 2 lung adenocarcinomas in which positivity was seen in about 70-80% of tumor cells, while some areas contained nodules of tumor cells that entirely lacked BAP1 staining.
**Calretinin was focally positive, i.e, 30% or less of tumor cells showed nuclear and cytoplasmic staining
***4 out of 15 cases of carcinosarcoma showed cytoplasmic staining but no nuclear staining for WT-1. WT-1 cytoplsmic staining is considered non-specific.
Among 13 CAM5.2 positive cases, 6 were focally positive on both the epitheliod and sarcomatoid components
All adenocarcinomas and squamous cell carcinomas were stained with TTF-1 NapsinA, CK5/6,P40, P63 to confirm diagnosis.

BAP1 mutations in sporadic Mesothelioma

- 4/18 (22%) – Testa JR et al, *Nature Genetics* 2011
- 12/53 (23%) – Bott M et al, *Nature Genetics* 2011
- 14/23 (61%) – Yoshikawa et al, *Cancer Science* 2012
- To address the discrepancy we conducted a joint study with the Japanese team and published 2 papers in JTO 2015 and PNAS 2016 addressing this issue

Journal of Thoracic Oncology, Volume 10, Number 4, April 2015

ORIGINAL ARTICLE

FAST TRACK

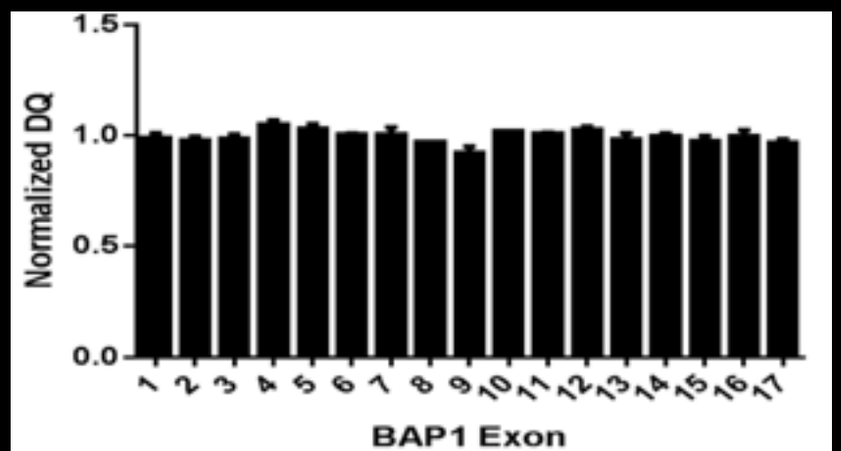
High Incidence of Somatic BAP1 Alterations in Sporadic Malignant Mesothelioma

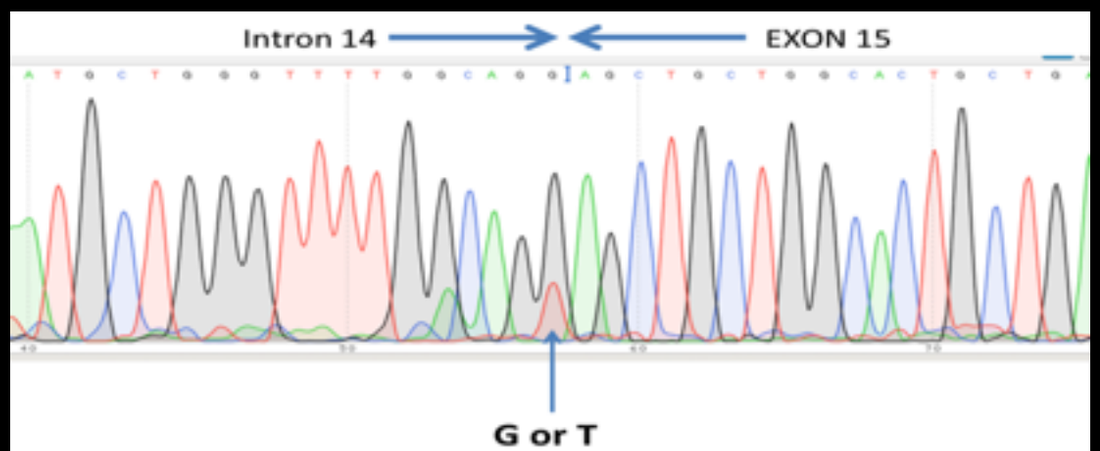
Masaki Nasu, Mitsuru Emi,* Sandra Pastorino,* Mika Tanji,* Amy Powers,* Hugh Luk,* Francine Baumann,* Yu-an Zhang,† Adi Gazdar,† Shreya Kanodia,*‡ Maarit Tiirikainen,* Erin Flores,* Giovanni Gaudino,* Michael J. Becich,§ Harvey I. Pass,‖ Haining Yang,* and Michele Carbone, MD, PhD**

Integrated genomic BAP1 analyses

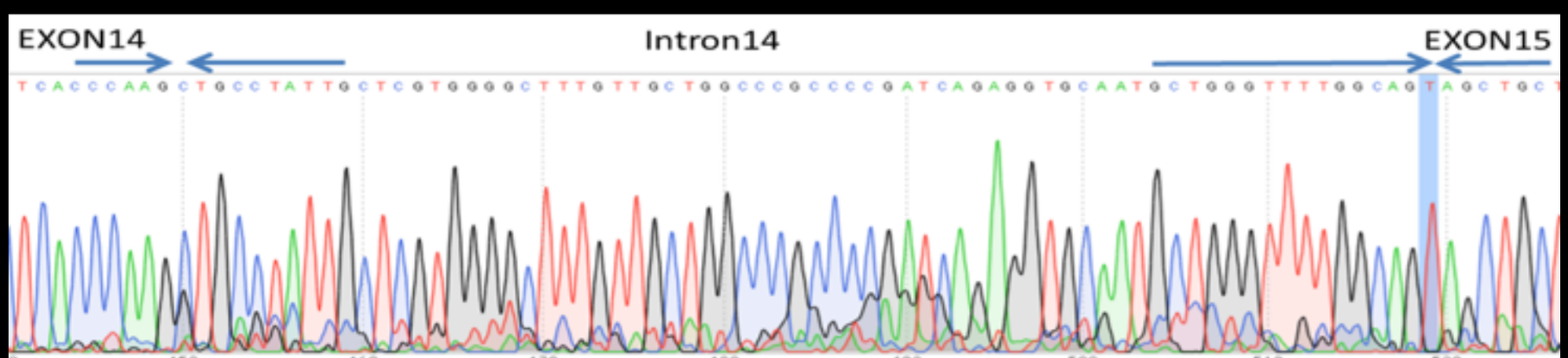
- From 22 laser-microdissected mesotheliomal biopsies and matching control tissue
  - Promoter methylation
  - Sanger Sequencing
  - MLPA
  - TaqMan copy number analyses
  - mRNA sequencing

Variety of BAP1 inactivation in somatic mesothelioma



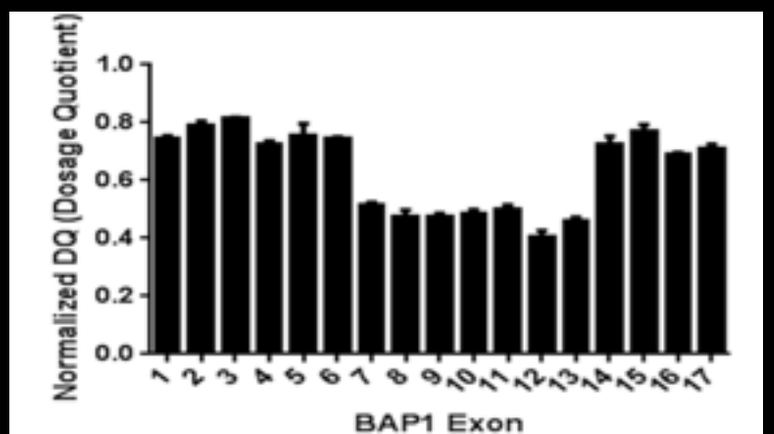


conventional Sanger sequencing. NYU 524

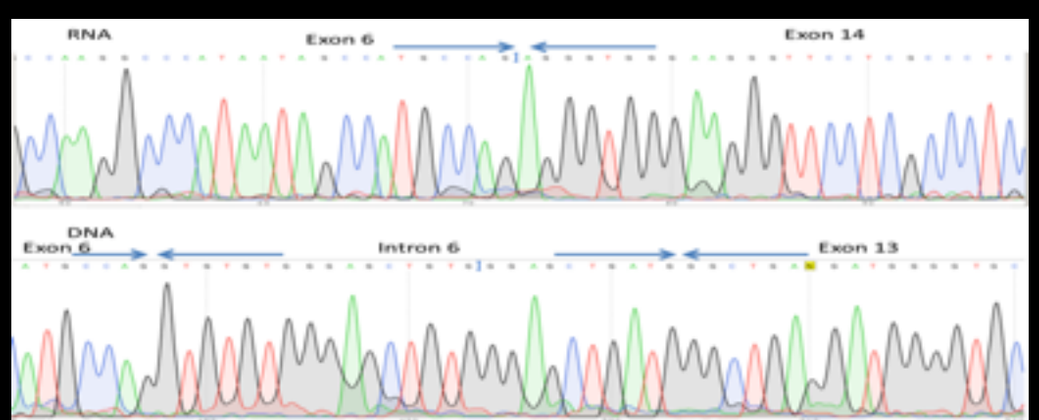


mRNA

Variety of BAP1 inactivation in somatic mesothelioma



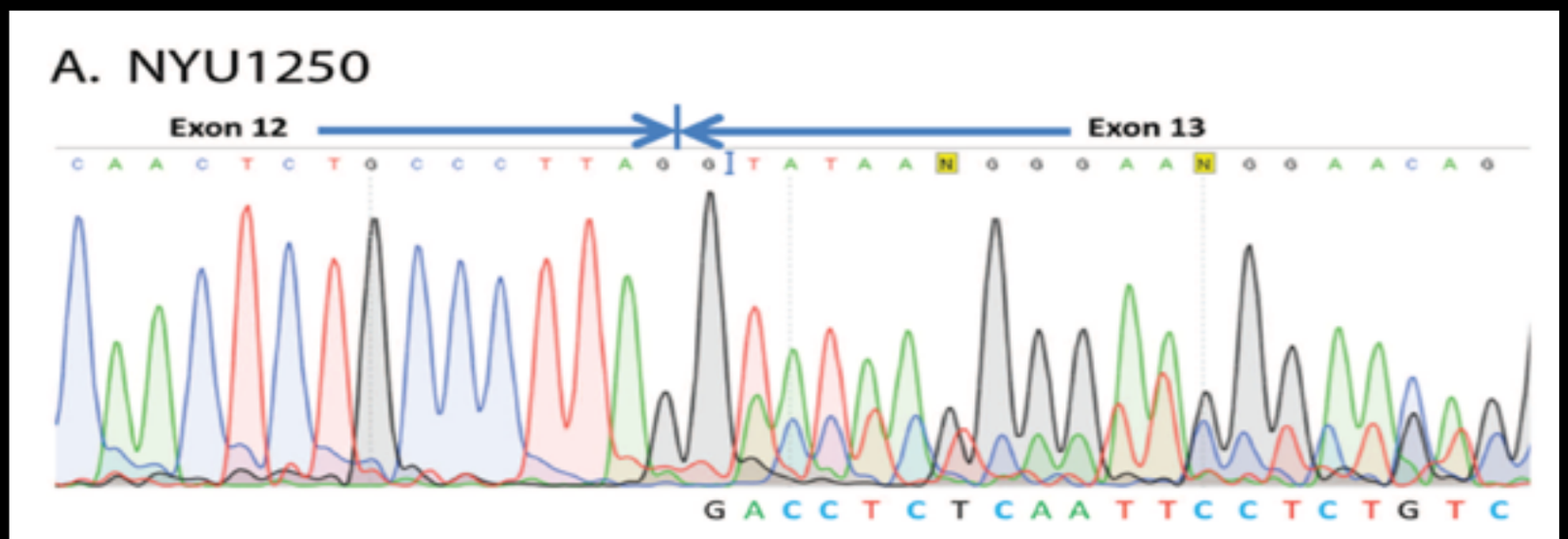
MLPA assay



RNA sequencing

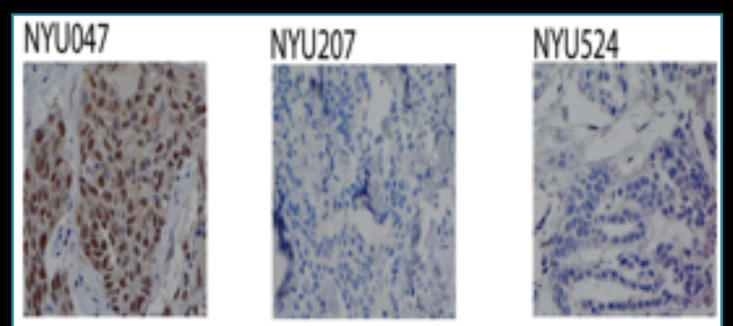
Genomic sequencing

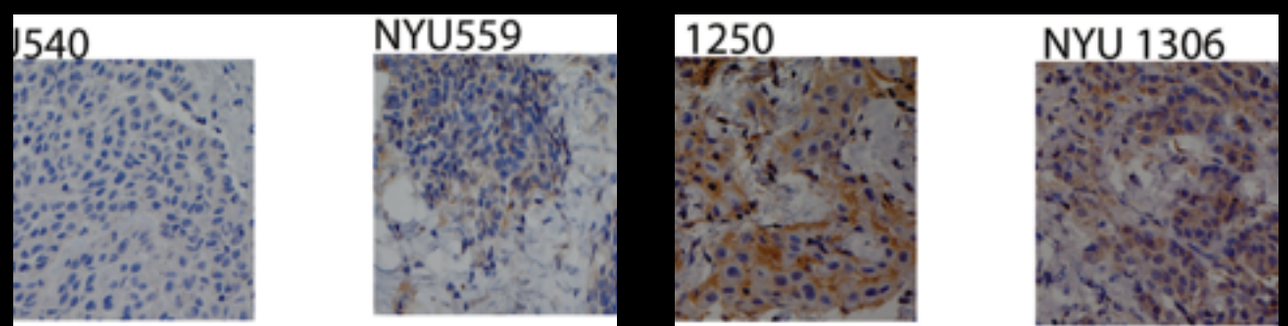
Abnormal splicing forms NYU 1250



ACCTGGCCCGTTCCCTTGCTTCACATCTTCTCGGGCCCCACAGGTATAAG
GGGAAGGGAACAGGGAAGCCAGGGGCATTGAGCGGTTCTGCTGATGGGCA
ACTGTCAGTGCTGCAGCCCAACACCATCAACGTCTTGGCTGAGAAGCTCA
AAGAGTCCCAGAAGGACCTCTCAATTCCTCTGTCCATCAAGACTAGCAGC
GGGGCTGGGAGTCCGGCTGTGGCAGTGCCCACACACTCGCAGCCCTCACC
CACCCCCAGCAATGAGAGTACAGACACGGCCTCTGAGATCGGCAGTGCTT
TCAACTCGCCACTGCGCT

Immunohistochemical staining to detect BAP1 mutations





Strong nuclear staining and weaker cytoplasmic staining indicates wild type BAP1

Absence of staining, or only cytoplasmic staining (which means mutated BAP1) , indicates biallelic BAP1 inactivating mutations.

PNAS

High-density array-CGH with targeted NGS unmask multiple noncontiguous minute deletions on chromosome 3p21 in mesothelioma

Yoshie Yoshikawa[a], Mitsuru Emi[a,b], Tomoko Hashimoto-Tamaoki[a,1], Masaki Ohmuraya[a], Ayuko Sato[c], Tohru Tsujimura[c], Seiki Hasegawa[d], Takashi Nakano[e], Masaki Nasu[b], Sandra Pastorino[b], Agata Szymiczek[b], Angela Bononi[b], Mika Tanji[b], Ian Pagano[b], Giovanni Gaudino[b], Andrea Napolitano[b], Chandra Goparaju[f], Harvey I. Pass[f], Haining Yang[b], and Michele Carbone[b,1]

[a]Department of Genetics, Hyogo College of Medicine, Nishinomiya, Hyogo J663-8501, Japan; [b]Thoracic Oncology Program, University of Hawaii Cancer Center, Honolulu, HI 96813; [c]Division of Molecular Pathology, Department of Pathology, Hyogo College of Medicine, Nishinomiya, Hyogo J663-8501, Japan; [d]Department of Thoracic Surgery, Hyogo College of Medicine, Nishinomiya, Hyogo J663-8501, Japan; [e]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo J663-8501, Japan; and [f]Department of Cardiothoracic Surgery, New York University Langone Medical Center, New York, NY 10016

Study Background

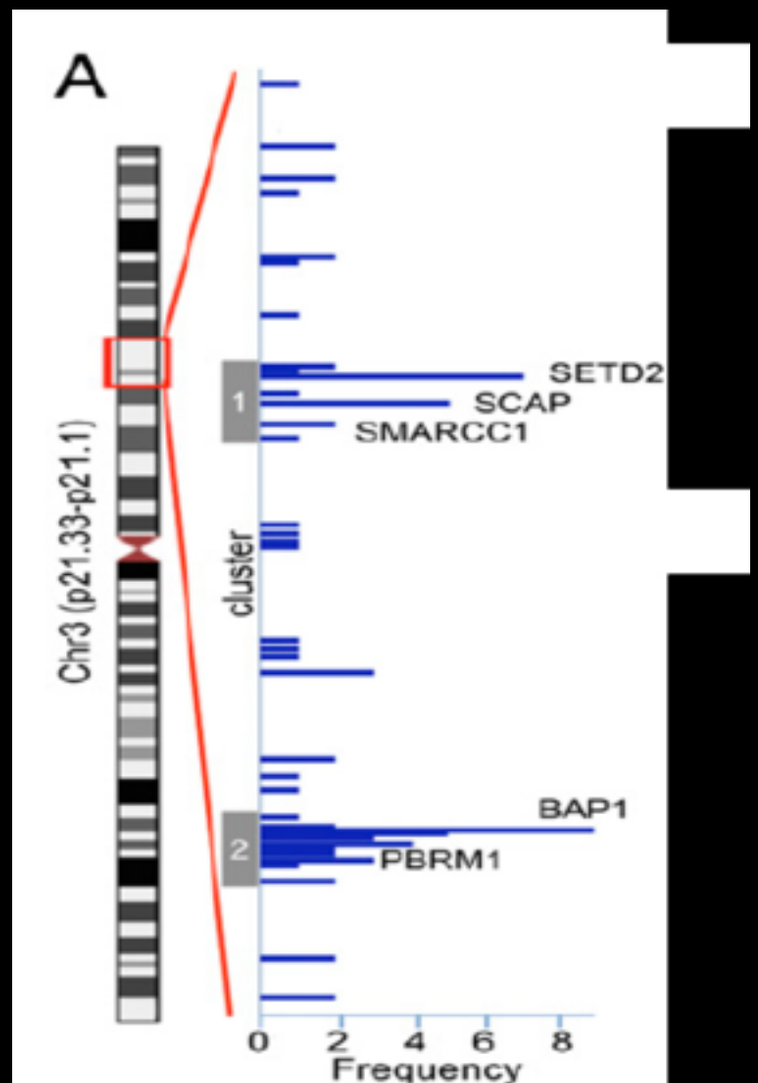
- NGS studies revealed that drivers mutations in mesothelioma, except for BAP1, CDKN2A and NF2 are rare
- Guo et al., Cancer Res 2015 conducted NGS exome studies on 22 MMs: total of 490 mutated genes: 97% mutated only in 1 MM.
- They found an average of mutations of 23 (2-51)
- Mutations in human cancer average 0.1/megabase in pediatric cancers to over 100/megabase in adult cancers
- Thus the low rate of mutations in mesothelioma, a tumor associated with a well known carcinogen, is very unusual
- Moreover, old cytogenetic studies revealed that MM had numerous numerical and structural chromosomal abnormalities, suggesting that genetic alterations in MM should be frequent

Study Background & Hypothesis

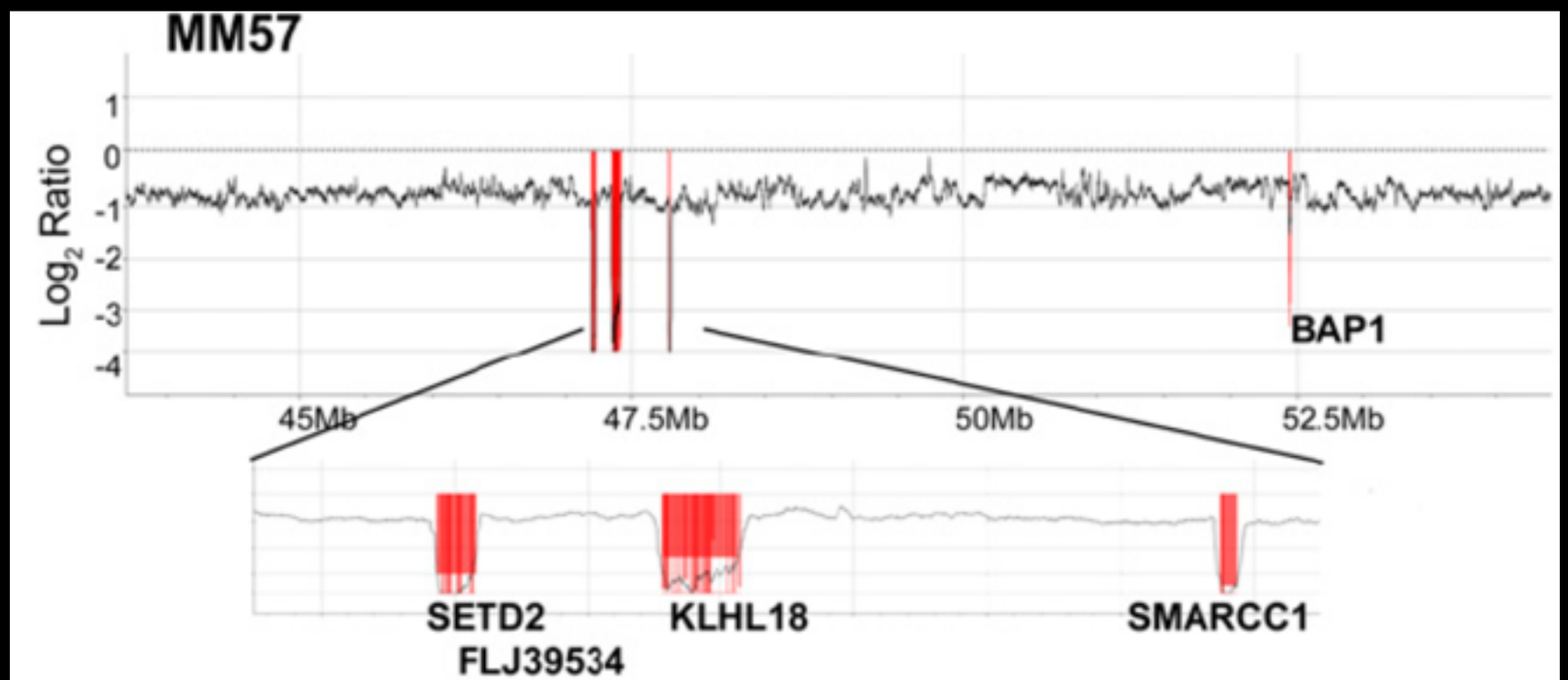
- Large DNA deletions are detected by whole genome aCGH which provide a coverage of about 1 probe every 3000-8000 bp (for example the Agilent CGH array), thus these array are not sensitive to detect minute DNA deletions (<3kb)
- NGS and tNGS are sensitive to detect minute deletions but they easily produce false positive results, because they are designed to detect nucleotide level mutations not CN changes
- We tested the hypothesis that MM contained minute DNA deletions that were missed by aCGH and NGS studies
- We designed a custom-made high density a-CGH (~1 probe/254bp) complemented with tNGS (> 150 reads)
- We focused on the 3p21 region, where BAP1 is located

Segmental copy number loss detected in 3p21 by high-density aCGH in representative MM

Frequency of biallelic deletion detected among 251 genes in 3p21 in 33 MMs



Genetic deletions on chromosome 3p in mesothelioma



Deletions are not contiguous: it's like the DNA was sliced with a knife

Conclusions

- Combined high-density aCGH and tNGS revealed biallelic gene inactivation in *SETD2* (9/33, 27%), *BAP1* (16/33, 48%), *PBRM1* (5/33, 15%) and *SMARCC1* (2/33, 6%).
- The incidence of genetic alterations detected is much higher than reported in the literature because minute deletions are not detected by NGS or commercial aCGH

Conclusions, continued

- Many of these minute deletions were not contiguous but rather they alternated with segments showing oscillating copy number changes along the 3p21 region as in chromothripsis.
- The 3p21 region contains a tumor suppressor gene cluster – RBM5, TUSC2, HYAL1, HYAL2- which are frequently deleted in human cancers. None of these gene contained biallelic deletions in MM.
- Thus inactivating mutations in 3p21 occur mostly in certain genes, and are not randomly distributed along the chromosome

Conclusions, continued

- Our results suggest that high-density aCGH combined with tNGS provide a more precise estimate of the frequency and types of genes inactivated in human cancer, than approaches based exclusively on NGS strategy
- Moreover, immunohistochemistry is a reliable and simple technique to identify BAP1 mutations

Project 2: Environmental Carcinogenesis

The roads of North Dakota

The gravel in Dunn County could be deadly.

Fear in the dust

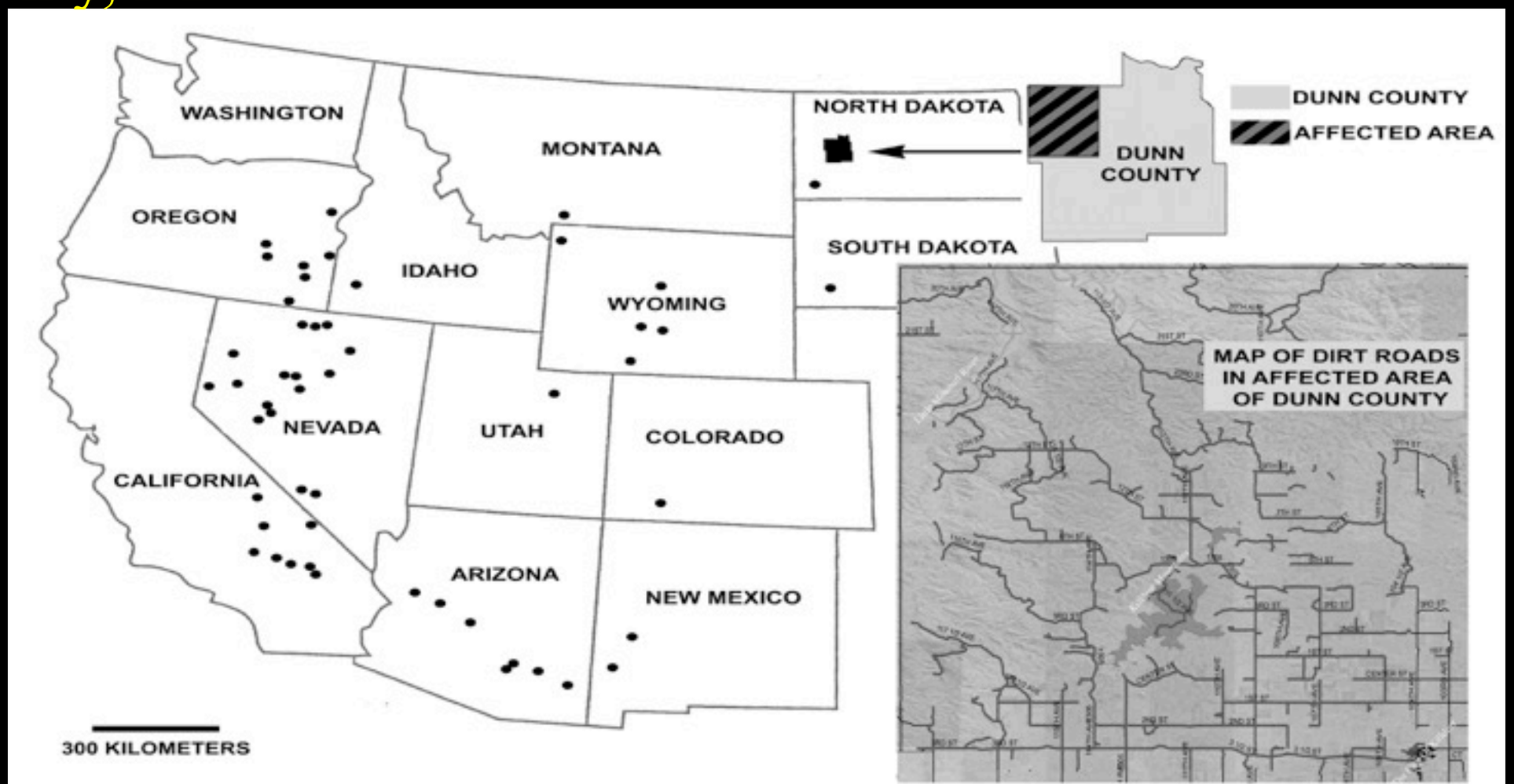
By Brendan Maher

Cancer epidemics in Turkey could hold the secret to staving off a public health disaster in North Dakota.

University of Hawaii in Honolulu who has worked in both North Dakota and the Turkish villages. "The reason that I find it exciting is that here we have a chance to do something," he says.

Carbone has been visiting Cappadocia since 1996, when he was invited by Izzettin Baris, a physician who at the time specialized in studying and

Geographic Distribution of Erionite in U.S.
We discovered >300 Miles of Erionite Gravel Roads in Dunn County, ND



Environmental exposure to carcinogenic mineral fibers is a GLOBAL problem

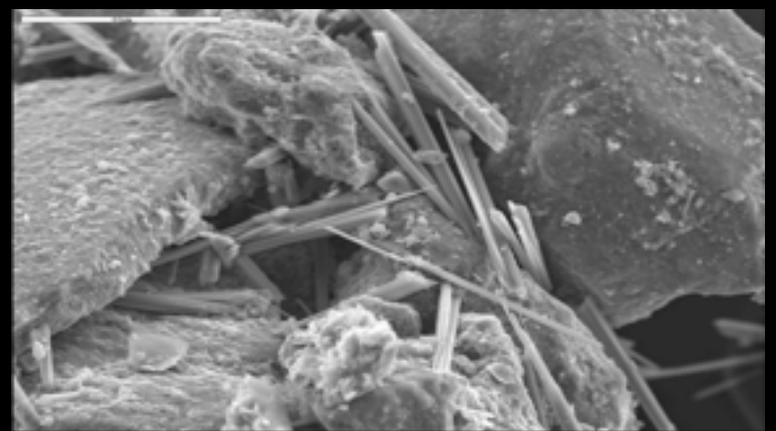
US – North Dakota

Exposure when:

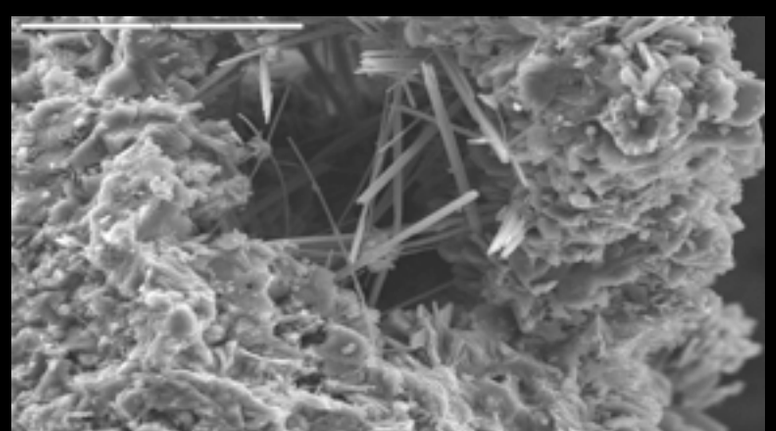
1. Fibrous minerals are present
2. Human activities or weathering release fibers
3. Human population is in contact with airborne fibers

Erionite mine in ND:
Our EPA collaborators taking samples

Characteristics of Erionite from North Dakota and Cappadocia are Similar



North Dakota Erionite



Cappadocia Erionite

		North Dakota (uM)	Turkey (uM)
Length	Mean	2.2	3.57
	Median	1.61	2.24
	Minimum	0.56	0.56
	Maximum	16.8	38.08
Width	Mean	0.31	0.31
	Median	0.28	0.2
	Minimum	0.05	0.06
	Maximum	1.28	5.04
Aspect Ratio	Mean	7.61	20.46
	Median	5.83	11.67
	Minimum	3	2.5
	Maximum	28.33	370

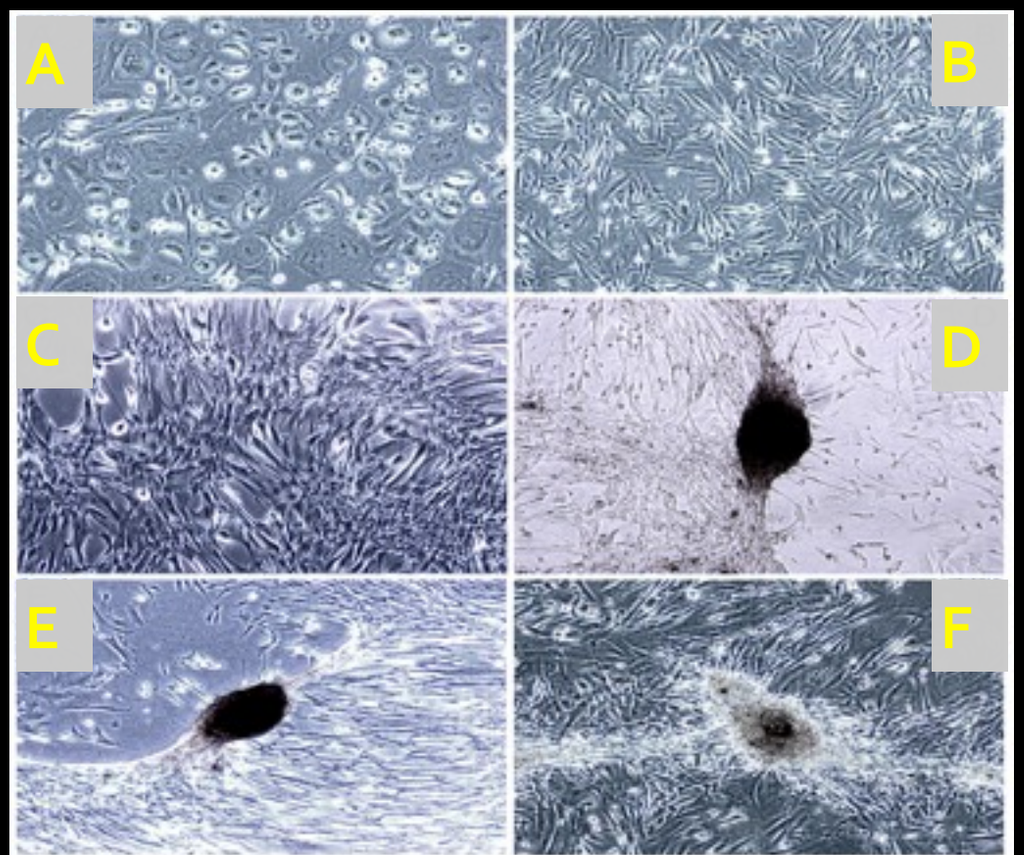
Aspect Ratio = Length:Width

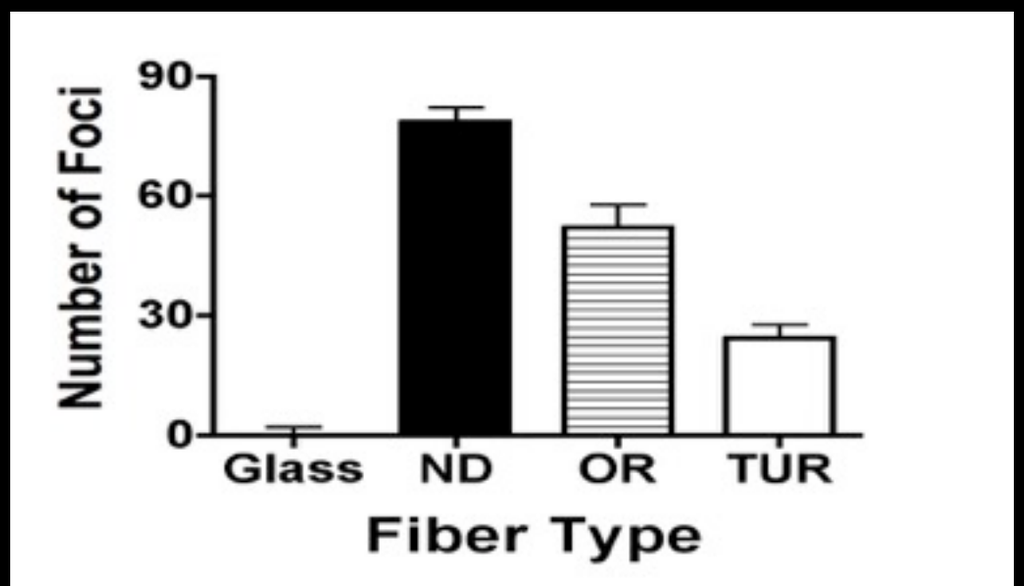
Maximum length is about 50 microns.

Widths are well below 1 micron.

Scale Bar: 50 microns

Carcinogenesis of Erionite Fibers from North Dakota, Oregon, and Cappadocia are Similar





Panel A Human Mesothelial Cells (HM) **Panel B** HM co-cultured with macrophages

Panel C-F HM co-cultured with macrophages and exposed to:
(**C**) Glass Fiber (**D**) North Dakota Erionite (**E**) Oregon Erionite (**F**) Cappadocia Erionite

Erionite Exposure in Dunn County, North Dakota
Air Concentrations. Red Circle: Air Concentration in School Bus

Scenario	Event	N Total	% Detect	Mean	Median	Range
Transportation ABS	TEM (s/cc)	41	90.24	0.235	0.036	2.74
	PCME (s/cc)	41	63.41	0.022	0.009	0.2
Transportation Stationary - adjacent to roadway	TEM (s/cc)	3	100.00	0.108	0.16	0.16
	PCME (s/cc)	3	100.00	0.012	0.014	0.02
Transportation Stationary - away from roadway	TEM (s/cc)	19	21.05	0.001	0	0
	PCME (s/cc)	19	10.53	0	0	0
Outdoor ABS	TEM (s/cc)	21	28.57	0.031	0	0.59
	PCME (s/cc)	21	14.29	0.003	0	0.05
Outdoor Stationary	TEM (s/cc)	29	6.90	0	0	0
	PCME (s/cc)	29	6.90	0	0	0
Indoor (Office) ABS	TEM (s/cc)	1	100.00	0.018	0.018	0
	PCME (s/cc)	1	0.00	0	0	0
Indoor (Office) Stationary	TEM (s/cc)	2	50.00	0.002	0.002	0
	PCME (s/cc)	2	50.00	0.001	0.001	0
Indoor (Garage) ABS	TEM (s/cc)	5	80.00	0.207	0.152	0.5
	PCME (s/cc)	5	40.00	0.061	0	0.17

Erionite Air Concentration in Turkish Villages

			Indoor Activity-Based Air Samples			Outdoor Street-side Stationary Air Samples			Outdoor Activity-Based Air Samples		
MM Mortality	Fiber	Analysis Method	N	% Detect	Mean	N	% Detect	Mean	N	% Detect	Mean
Karain, Turkey											
51.50%	Erionite	TEM (s/cc)	9	100	7.817	8	0	0	6	66.6	0.0856
		PCME (s/cc)	9	77.7	1.737	8	0	0	6	66.6	0.0106
	Total Asbestos	TEM (s/cc)	9	55.5	0.167	8	0	0	6	16.6	0.0024
		PCME (s/cc)	9	0	0	8	0	0	6	16.6	0.0024
Sarihidir, Turkey											
38.20%	Erionite	TEM (s/cc)	10	100	3.589	7	57	0.0028	8	62.5	0.3739
		PCME (s/cc)	10	100	0.684	7	57	0.0017	8	50	0.1398
	Total Asbestos	TEM (s/cc)	10	90	0.419	7	14	0.0004	8	37.5	0.2097
		PCME (s/cc)	10	0	0	7	0	0	8	12.5	0.0262
Tuzkoy, Turkey											
25.90%	Erionite	TEM (s/cc)	5	100	7.324	4	25	0.0091	5	100	0.2854
		PCME (s/cc)	5	80	1.107	4	0	0	5	60	0.0562
	Total Asbestos	TEM (s/cc)	5	100	2.026	4	25	0.0167	5	80	3.6421
		PCME (s/cc)	5	20	0.053	4	25	0.0015	5	40	0.1556
Karlik, Turkey											
7.41%	Erionite	TEM (s/cc)	5	100	0.2221	4	50	0.0038	4	75	0.4464
		PCME (s/cc)	5	40	0.0157	4	0	0	4	50	0.0552
	Total Asbestos	TEM (s/cc)	5	80	0.1084	4	0	0	4	75	0.0834
		PCME (s/cc)	5	0	0	4	0	0	4	0	0
Boyali, Turkey											
6.25%	Erionite	TEM (s/cc)	3	100	0.0431	5	0	0	4	75	0.0411
		PCME (s/cc)	3	100	0	5	0	0	4	0	0
	Total Asbestos	TEM (s/cc)	3	0	0	5	20	0.0001	4	75	0.0515
		PCME (s/cc)	3	0	0	5	0	0	4	0	0
Dunn County, North Dakota (*12 per million in ND)											
Unknown*	Erionite	TEM (s/cc)	6	83	0.175	3	100	0.1082	20	25	0.0031
		PCME (s/cc)	6	33	0.0575	3	100	0.0122	20	10	0.0004
	Total Asbestos	TEM (s/cc)	6	50	0.3054	3	0	0	20	0	0
		PCME (s/cc)	6	0	0	3	0	0	20	0	0

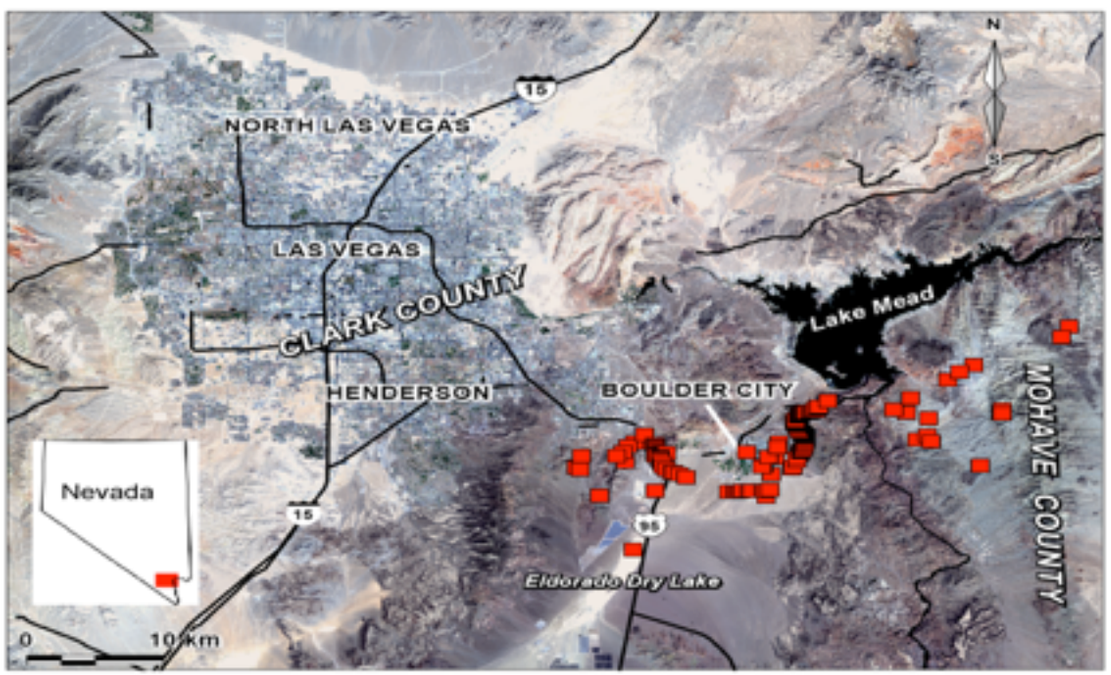
In response to our paper, roads were re-paved with erionite free gravel –red gravel- saving many future lives to mesothelioma

Carbone M et al. Erionite exposure in North Dakota and Turkish villages with mesothelioma. *Proc Natl Acad Sci USA*, 108:13618-23, **2011.**

Increased MM in some US states

- Increased Proportion of Women and Young Mesothelioma cases in Southern Nevada: Indicators of Possible Environmental Exposure to Carcinogenic Fibrous Minerals – Journal of Thoracic Oncology, 10:731-7, 2015
  - Baumann F,…Carbone M

High incidence of MM in young people in Nevada



Extensive deposits of asbestos in the ground in area around Las Vegas

Dust storm carries asbestos

Insert figure showing SEM image of fibrous amphiboles from Black Hill, Henderson in this photos of a dust storm entering Henerson-Las Vegas, obscuring the McCullough Mountains (October 19, 2009)

Peritoneal mesothelioma among young women in China

Letters

RESEARCH LETTER

Association of Asbestos Exposure With Malignant Mesothelioma Incidence in Eastern China

JAMA Oncology, April 2017, Volume 3, Number 4

Improving the accuracy of mesothelioma diagnosis in China

- Zhenying Guo[1]*, Michele Carbone[2*#], Xing Zhang[6], Dan Su[3,4], Wenyong Sun[1], Jianlin Lou[6], Zhibin Gao[7], Dichu Shao[8], Junqiang Chen[6], Gu Zhang[1], Jinlin Hu[1], Kaiyan Chen[3,4], Fang Wang[9], Harvey I. Pass[10], Herbert Yu[11], Andrea Napolitano[2], Haining Yang[2] and Weiming Mao[3,4,5,#]

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- 2. Thoracic Oncology, University of Hawaii Cancer Center, Honolulu, USA
- 3. Cancer Research Institute, Zhejiang Cancer Hospital , Hangzhou, China.
- 4. Key Laboratory Diagnosis and Treatment Technology on Thoracic Oncology of Zhejiang Province, Hangzhou, China.
- 5. Department of Thoracic Surgery, Zhejiang Cancer Hospital, Hangzhou, China
- 6. Institute of Occupational Diseases, Zhejiang Academy of Medical Sciences, Hangzhou, China
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- 8. Yuyao Center of Disease Control and Prevention, Yuyao, Zhejiang, China
- 9. Department of Radiology, Zhejiang Cancer Hospital, Hangzhou, China.
- 10. Department of Cardiothoracic Surgery, New York University Langone Medical Center, New York, USA
- 11. Epidemiology Program, University of Hawaii Cancer Center, Honolulu, USA
- *Equally contributed to this manuscript; #co-corresponding authors

JTO, December 2016

Findings

- The reported incidence of malignant mesothelioma (MM) in China is 1.5 cases/million, much lower than in other countries. In China, the prevalence of MM in different regions is unknown, and only 15% of MMs have been associated with asbestos exposure compared to over 70% in most of the world.
- During my visit to the Zhejiang Cancer Hospital (ZJCH), which is the largest Cancer Hospital in the city of Hangzhou where there is no asbestos industry, I was asked for a consult because a patient had just been diagnosed with MM. It was not a MM.
- We reviewed all pleural and peritoneal malignancies diagnosed as MM during the years 2002-2015 in ZJCH, and in the nearby (112 km) Yuyao People's Hospital, located in the Chinese textile asbestos industrial area, where most patients are exposed to asbestos. There were a total of 92 MMs: 34 in the pleura, 56 in the peritoneum, 2 in the tunica vaginalis.

Findings, continued

- Reviewing histology with the aid of a large panel of immunohistochemistry we confirmed the diagnosis in 28 MMs from ZJCC and 24 from Yuyao Hospital, 52/92 cases (56.5%).
- The M:F ratio was approximately 1:4 (19.2%, 95% CI: 9.6%–32.5%) compared to 4:1 (80%) in the US ($z = 11.0$, $p < .0001$).
- The pleural:peritoneal ratio was approximately 1:3 (24.0%, 95% CI: 13.1%–38.2%) compared to 5:1 (83.3%) in the US ($z = 11.2$, $p < .0001$).
- The average age of diagnosis was 50.6 (95% CI: 48.4–52.7) compared to 72 in the US [$t(51) = 19.3$, $p < .0001$].
- 9/52 (17.3%, 95% CI: 8.2%–30.3%) MMs occurred in individuals aged 40 or younger, compared to less than 1% in the same age group in the US.

Findings, continued

- Asbestos exposure was determined by a trained oncologist who interviewed patients for occupational and family histories.
- Individuals who worked in trades where asbestos exposure was likely, or who grew up/lived in families in which others were employed in the textile industry or trades associated with asbestos, were identified as "asbestos-exposed."
- 20/52 MMs (5 in the pleura, 14 in the peritoneum and 1 in the tunica vaginalis) occurred among asbestos-exposed individuals, (38.5%, 95% CI: 25.3%–53.0%): 2/28 (7.1%, 95% CI: 0.9%–23.4%) from ZJCC and 18/24 (75.0%, 95% CI: 53.3%–90.2%) from Yuyao Hospital.
- CT scans available for 16/28 ZJCC MMs supported asbestos exposure in 2/16 (12.5%, 95% CI: 1.6%–38.4%). Asbestos-related MMs included 19 women and one man.

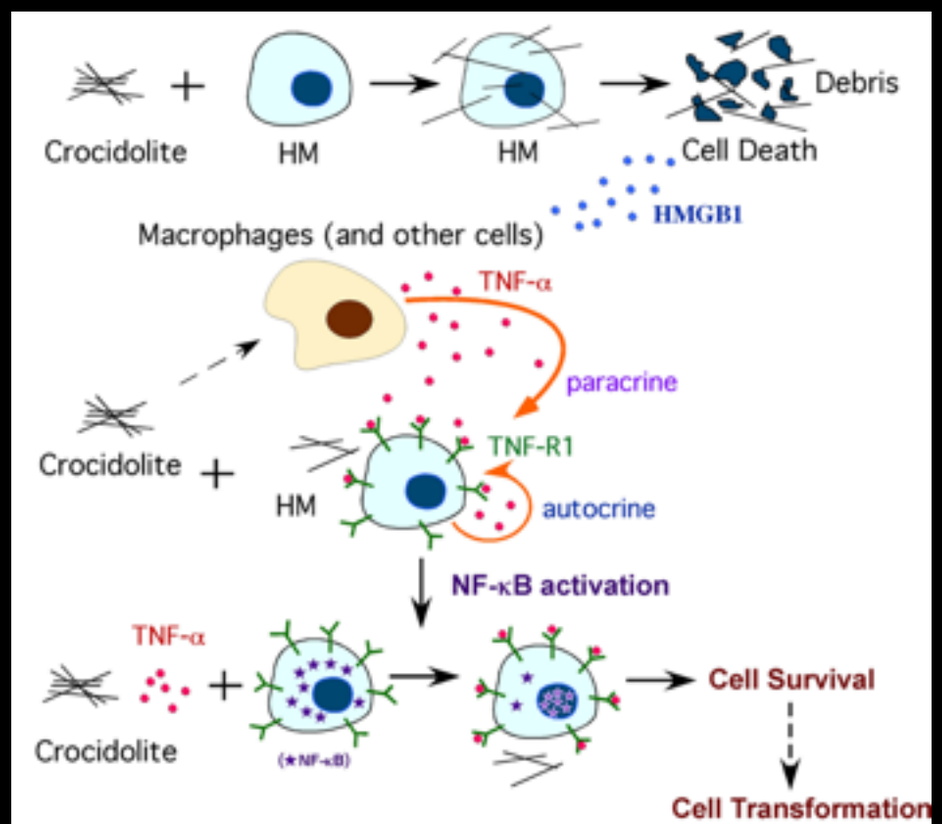
Conclusions

- The higher prevalence of peritoneal versus pleural MMs in China, especially among non-exposed young women, is unique. Initially we thought that asbestos was the main cause, as 13/18 (72.2%, 95% CI: 46.5%–90.3%) peritoneal MMs from Yuyao-Hospital occurred in women exposed to asbestos. However, only 1/14 (7.1%, 95% CI: 0.2%–33.8%) peritoneal MM from ZJCC was associated with asbestos.
- Except for asbestos exposure, no significant demographic differences were observed between ZJCC and Yuyao Hospitals (Tables1-2), suggesting that asbestos may not be the main cause of MM in these women.

Conclusions, continued

- In the US and Europe, an increasing number of peritoneal MMs do not seem associated with asbestos, leading some Authors to speculate that a sub-group of peritoneal MMs may have a different pathogenesis.
- Our findings point to a unique opportunity to investigate other causes of peritoneal MMs in this Chinese population, aside from asbestos, and when identified to implement preventive measure to decrease the incidence in future generations.

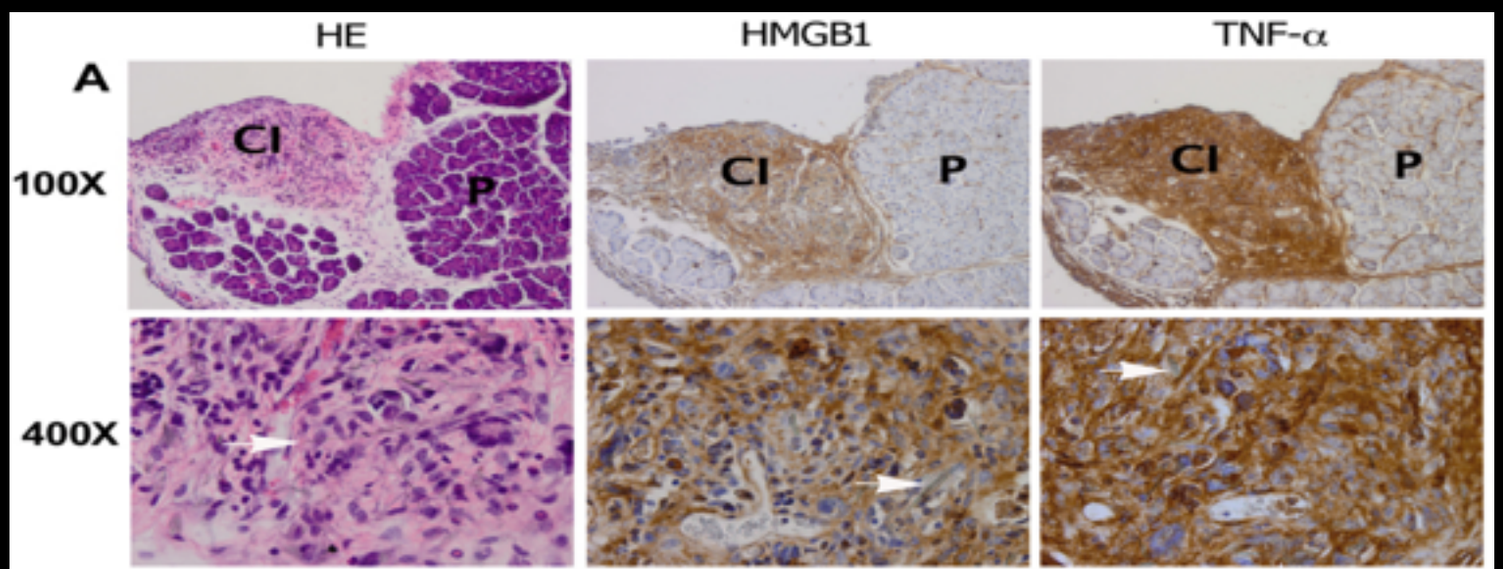
Research Project 3: Asbestos Carcinogenesis



HMGB1 as a mechanism of Asbestos Carcinogenesis

- PNAS articles by Yang H et al, 2006 and 2010
- HMGB1 is mostly present in the nucleus of the cell
  - It stabilizes the DNA
- When cells die, they passively release HMGB1
- Cells can also actively secrete HMGB1
  - HMGB1 needs to be acetylated for release, preventing the HMGB1 protein from moving from the cytoplasm to the nucleus

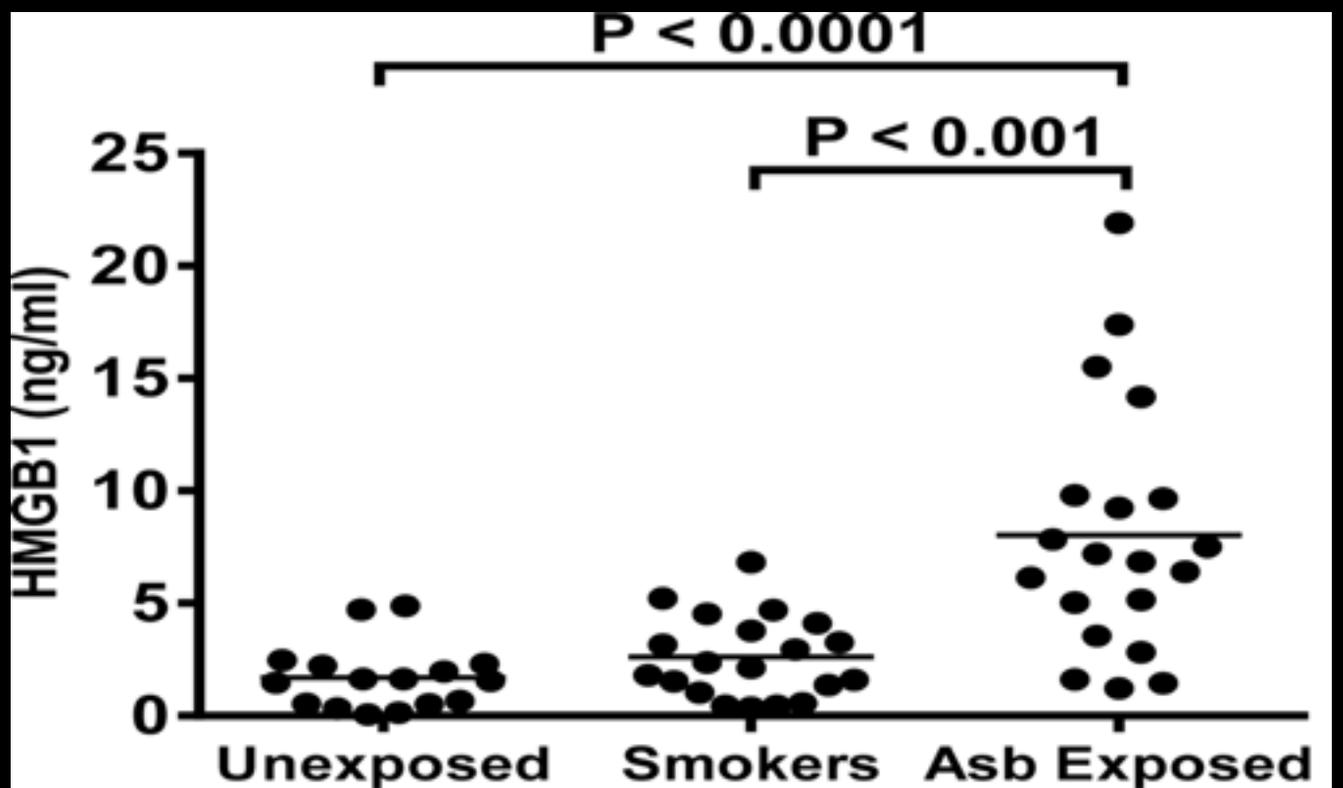
HMGB1 at sites of asbestos deposits in mice



Strong HMGB1 and TNF-α staining around areas of asbestos deposits

- In animals injected with asbestos, HMGB1 was detected specifically at the site of asbestos deposits, together with TNF-alpha, whose production is triggered by HMGB1
- The brown color identifies these proteins by immunohistochemistry

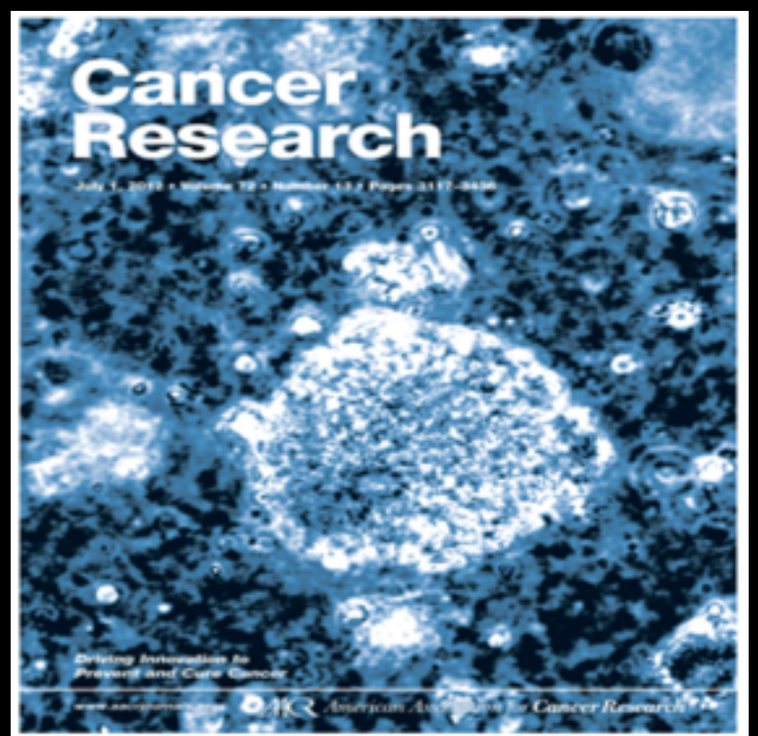
Serum HMGB1 levels in individuals exposed to asbestos, in heavy smokers, and in controls



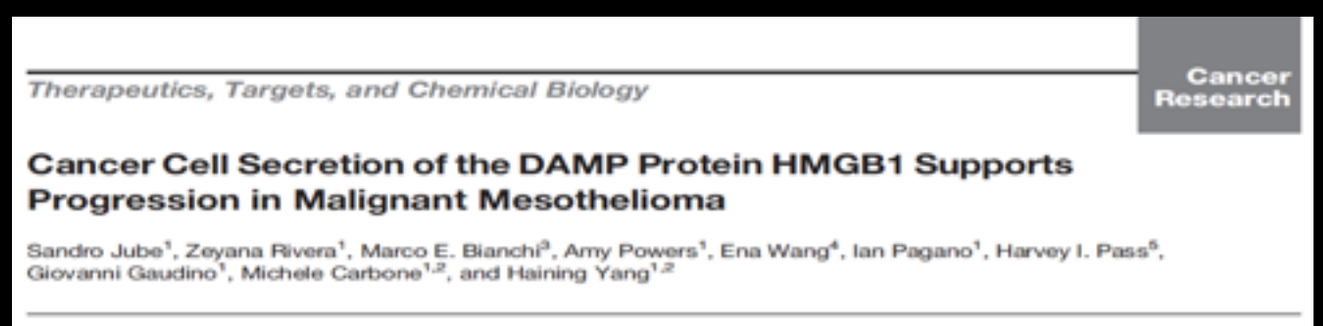
Yang H et al, PNAS 2010

The sera of individuals exposed to asbestos has high levels of HMGB1

Mesothelioma cells grow from an environment rich in HMGB1 and need it for their growth



The cover of the July 2012 issue of Cancer Research illustrates our discovery of how HMGB1 is integral to mesothelioma development



Therapeutics, Targets, and Chemical Biology

Cancer Research

Cancer Cell Secretion of the DAMP Protein HMGB1 Supports Progression in Malignant Mesothelioma

Sandro Jube[1], Zeyana Rivera[1], Marco E. Bianchi[3], Amy Powers[1], Ena Wang[4], Ian Pagano[1], Harvey I. Pass[5], Giovanni Gaudino[1], Michele Carbone[1,2], and Haining Yang[1,2]

Yang H et al, Cancer Research July 2012

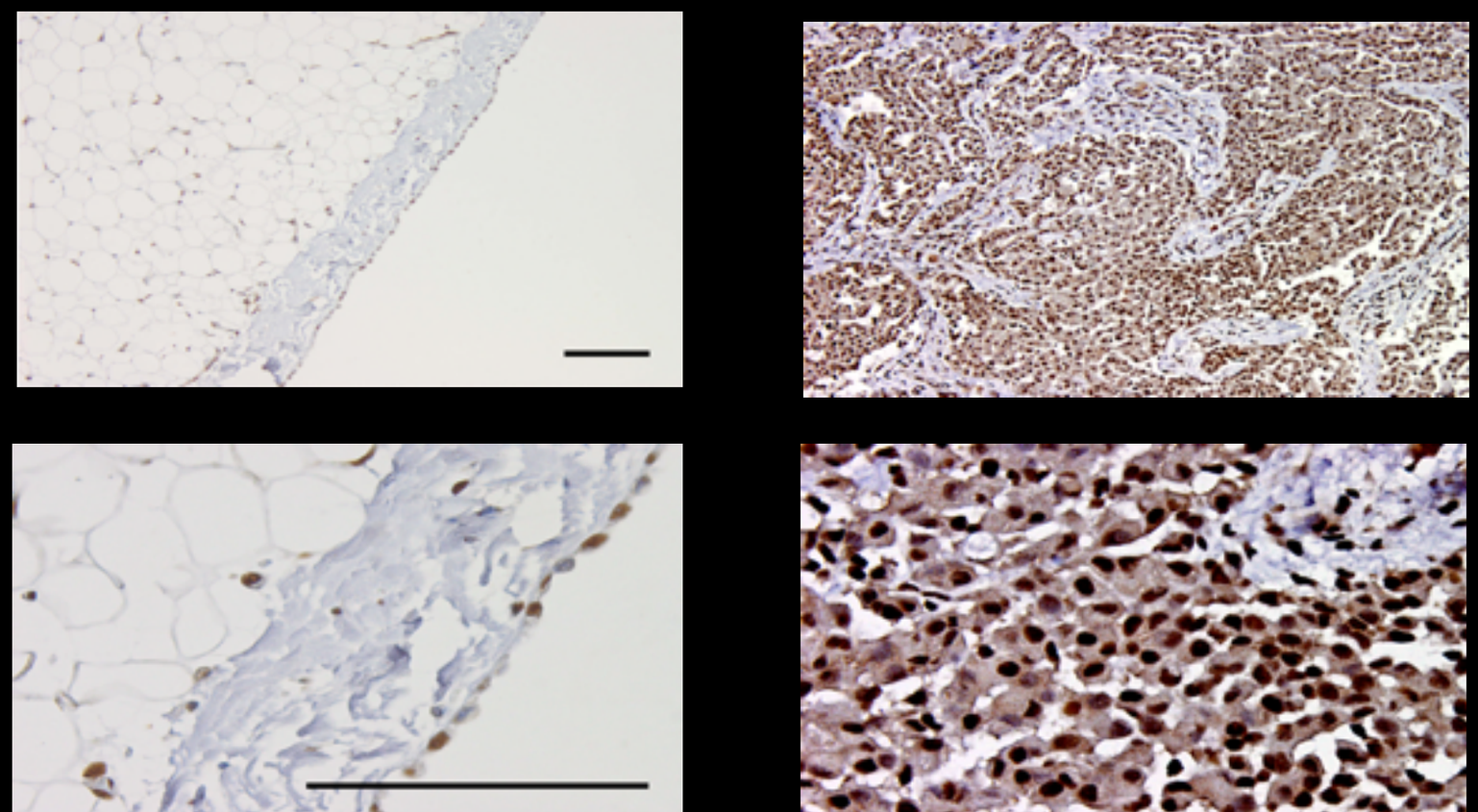
HMGB1 is highly expressed in mesothelioma tissue

Normal Pleura

MM Pleura

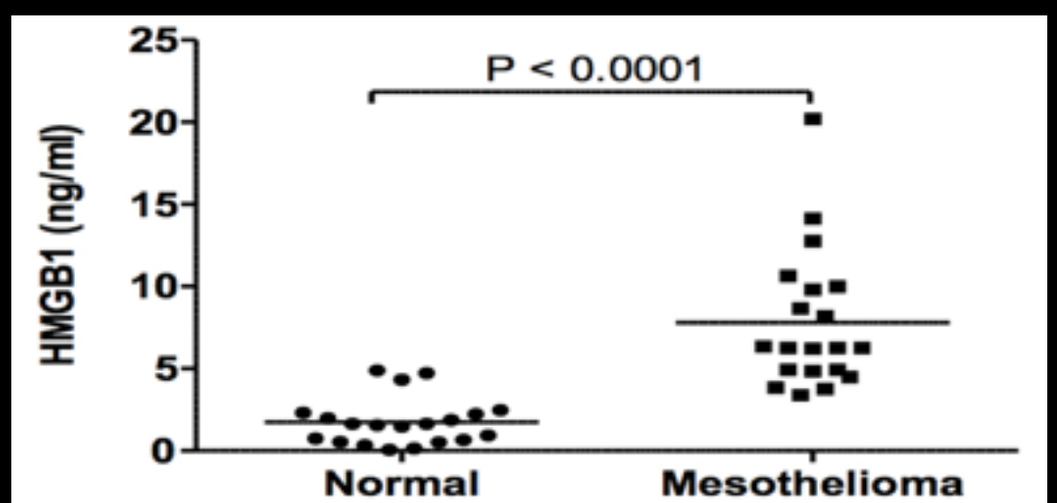
100X

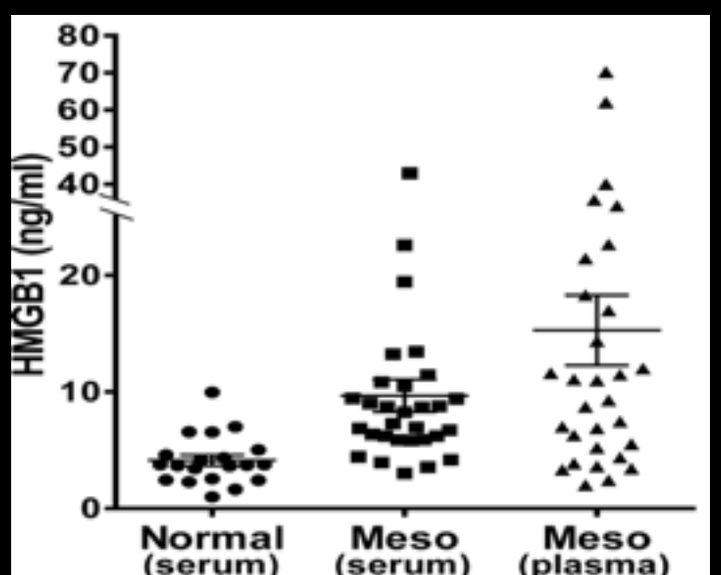
400X



Yang H et al, Cancer Research July 2012

HMGB1 serum level is significantly higher in MM patients than in healthy controls





Mesothelioma screening program

Aspirin reduces HMGB1 serum levels

Cell Death & Disease

Original Article

Citation: *Cell Death and Disease* (2015) **6**, e1786; doi:10.1038/cddis.2015.153
Published online 11 June 2015

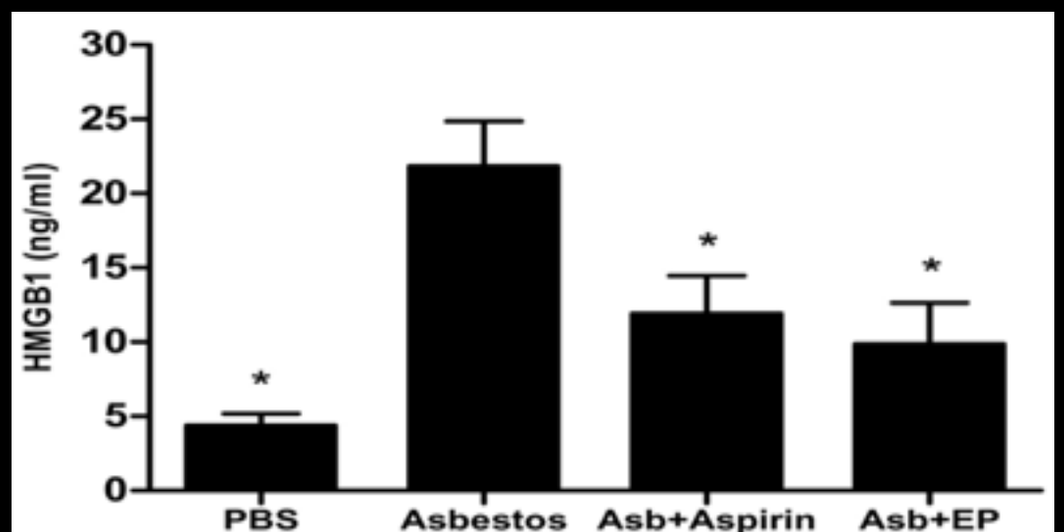
Aspirin delays mesothelioma growth by inhibiting HMGB1-mediated tumor progression

OPEN

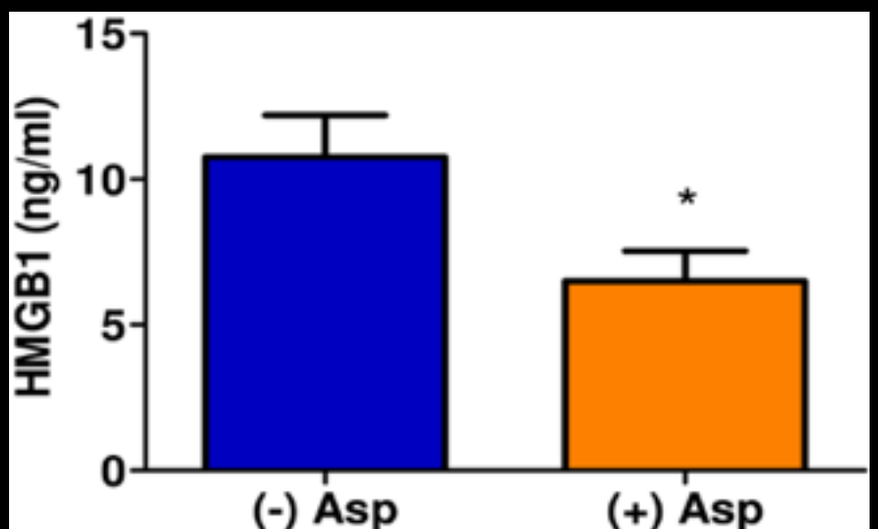
H Yang[1], L Pellegrini[1], A Napolitano[1,2], C Giorgi[3], S Jube[1], A Preti[4], C J Jennings[1], F De Marchis[4], E G Flores[1], D Larson[1], I Pagano[1], M Tanji[1], A Powers[1], S Kanodia[5], G Gaudino[1], S Pastorino[1], H I Pass[6], P Pinton[3], M E Bianchi[4] and M Carbone[1]

Cell Death and Disease 2015

Aspirin reduces HMGB1 serum levels

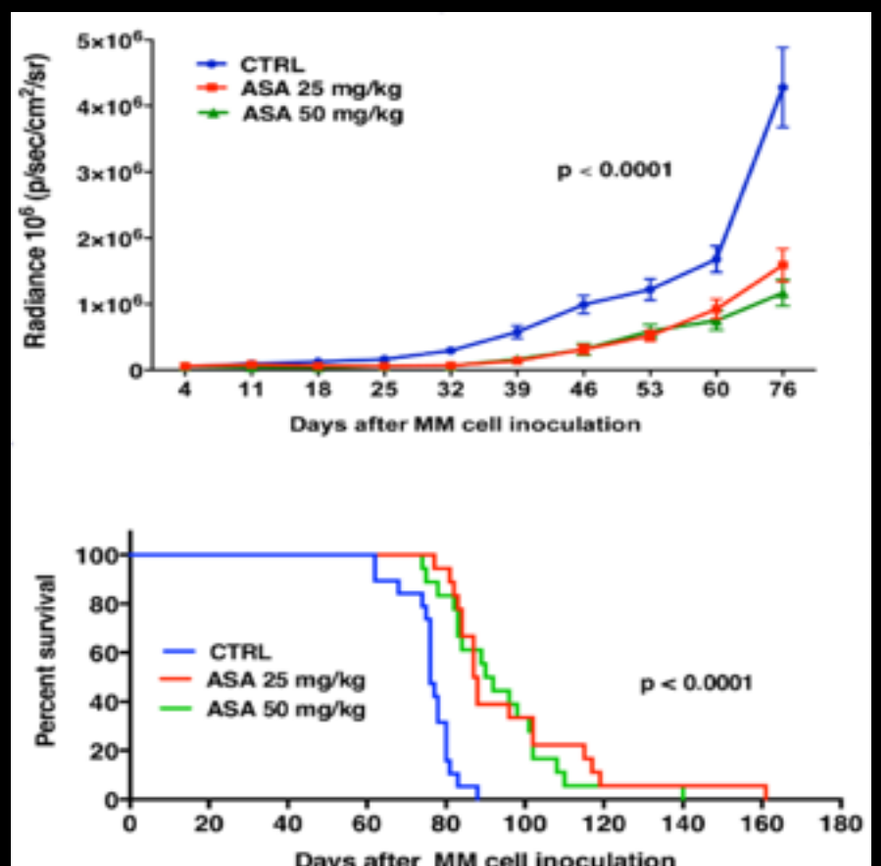


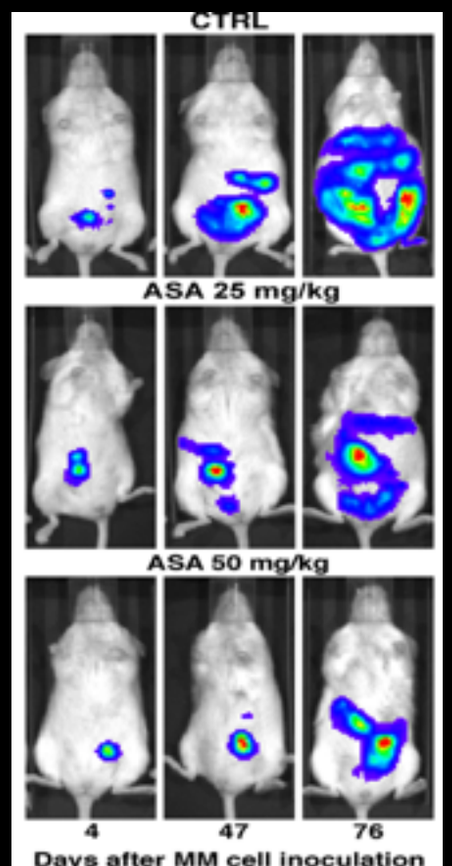
Aspirin reduces HMGB1 serum levels in mice exposed to asbestos



Aspirin reduces HMGB1 serum levels in mice with MM

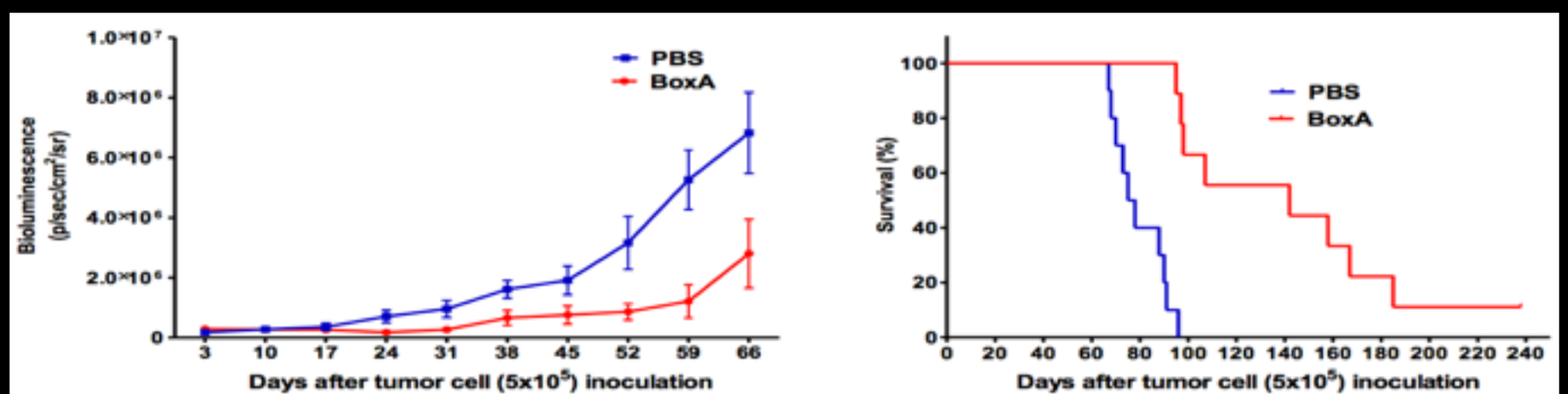
Aspirin inhibits MM tumor growth in mice





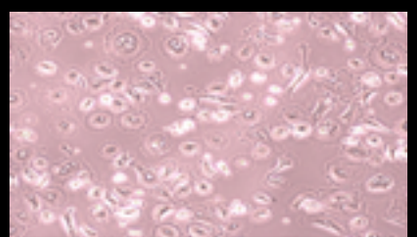
- Aspirin treatment reduces the growth of human mesothelial cells injected intraperitoneally in mice
- Aspirin prolongs survival

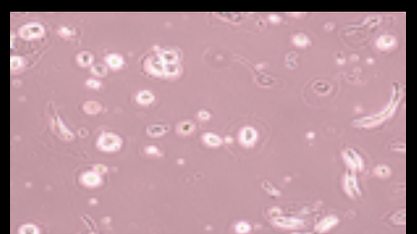
Targeting HMGB1 with BOX A inhibitor may be a possible therapeutic strategy for MM



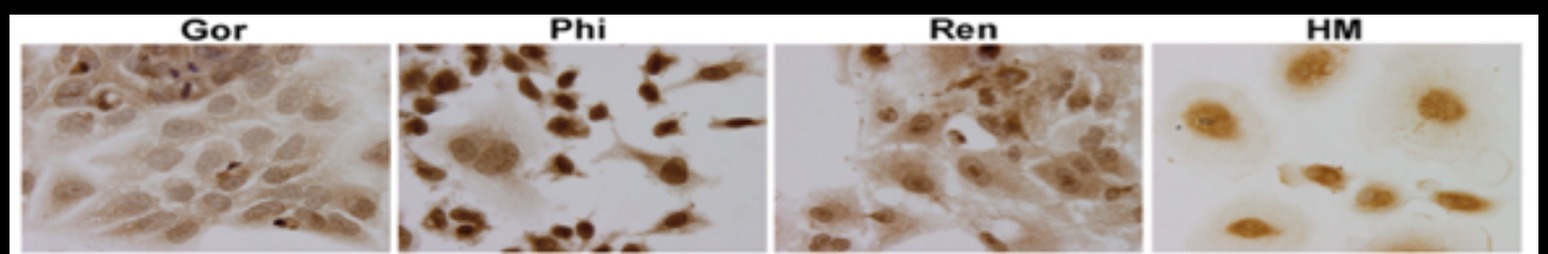
BOX A, a specific HMGB1 inhibitor, reduces the growth of human mesothelioma cells injected in mice and prolongs survival

HMGB1 Isoforms

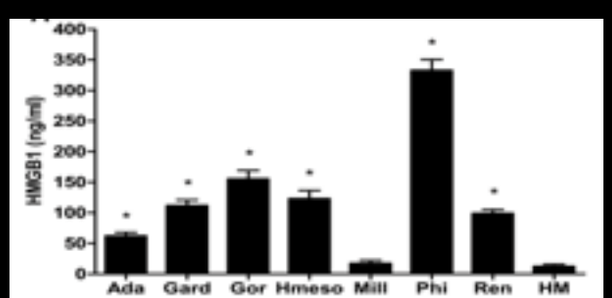




Asbestos-exposed human mesothelioma cells
→ HMGB1 is released **passively** (**non-acetylated**)



Mesothelioma cells instead actively secrete acetylated HMGB1



Human MM cells secrete HMGB1 **actively** (**hyper-acetylated**)

Total and hyper-acetylated HMGB1 are biomarkers for asbestos exposure and MM

Published OnlineFirst January 5, 2016; DOI: 10.1158/1078-0432.CCR-15-1130

Biology of Human Tumors

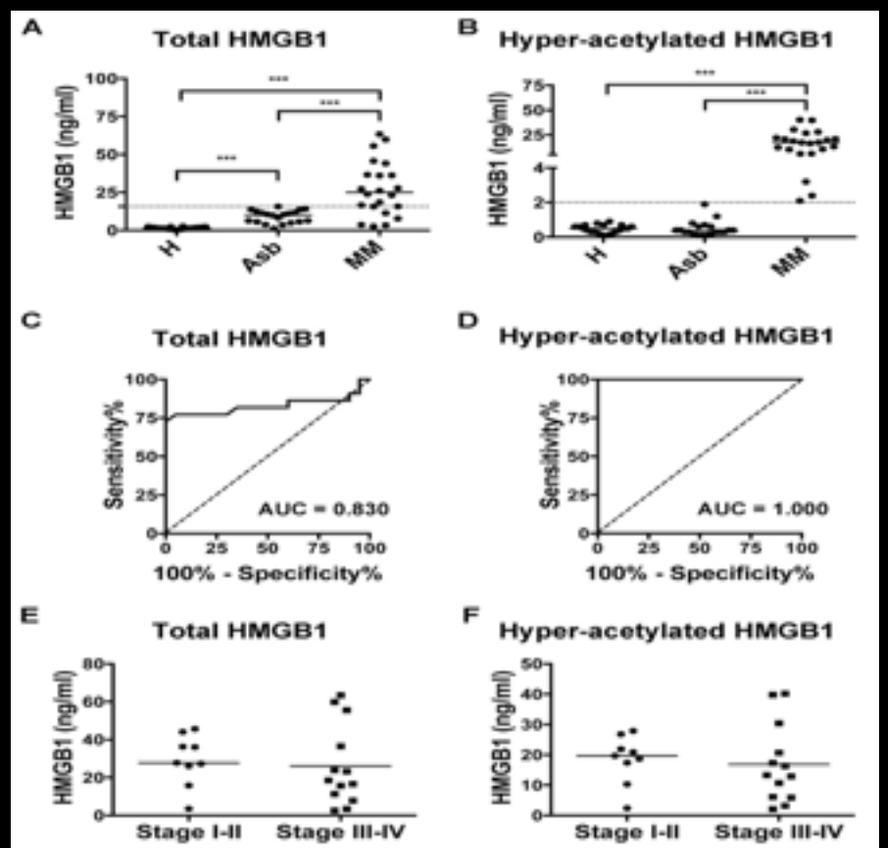
Clinical Cancer Research

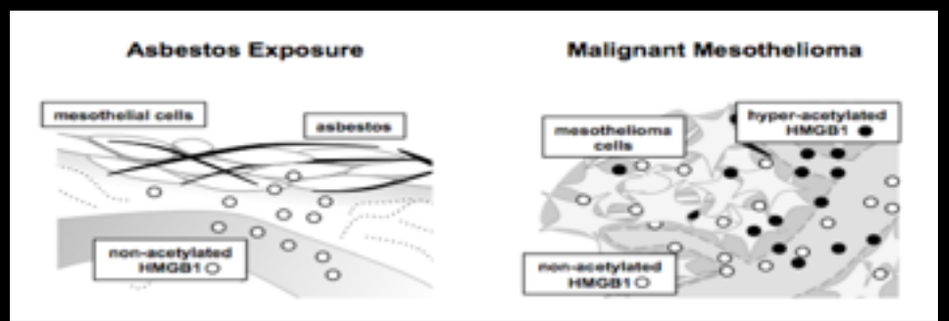
HMGB1 and Its Hyperacetylated Isoform are Sensitive and Specific Serum Biomarkers to Detect Asbestos Exposure and to Identify Mesothelioma Patients

Andrea Napolitano[1,2], Daniel J. Antoine[3], Laura Pellegrini[1], Francine Baumann[1], Ian Pagano[1], Sandra Pastorino[1], Chandra M. Goparaju[4], Kirill Prokrym[4], Claudia Canino[4], Harvey I. Pass[4], Michele Carbone[1], and Haining Yang[1]

HMGB1 and Its Hyperacetylated Isoform are Sensitive and Specific Serum Biomarkers to Detect Asbestos Exposure and to Identify Mesothelioma Patients, Clinical Cancer Research, June 2016

Findings





- We can identify MM patients from individuals exposed to asbestos from non-exposed individuals by measuring total levels of HMGB1 and the different isoforms, acetylated and non acetylated, present in their serum.
- A clinical trial is ongoing to validate these findings.

Conclusions: HMGB1 and MM

- HMGB1 plays a critical role in asbestos carcinogenesis and MM pathogenesis.
- Targeting HMGB1 can be a novel strategy for MM prevention and therapy
- Total HMGB1 and its hyper-acetylated isoform can be good biomarkers for asbestos exposure and mesothelioma detection.
- A clinical trial in larger cohorts will be conducted to validate the findings.
- MM early detection screening program in BAP1 mutant carriers ("BAP1 cancer syndrome") and high-risk asbestos-exposed cohorts.

Mahalo from our research lab team!