@AsbestosSafety Asbestos Safety and Eradication Agency asbestossafety.gov.au/asbestosconference2018

ASBESTOS: the next national plan

Proactivity, prevention, planning

Workshop 5

Research Directions

#2018ASEACONF

Australian Government
Asbestos Safety and Eradication Agency

NSW

Thuroona Services

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the next national plan

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Welcome

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Australian Government
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NSW

Thuroona Services

From Bench to Public: Another Direction of Translational Research

Ken Takahashi, Yuen Cheng, Matthew Soeberg
Asbestos Diseases Research Institute

Introduction

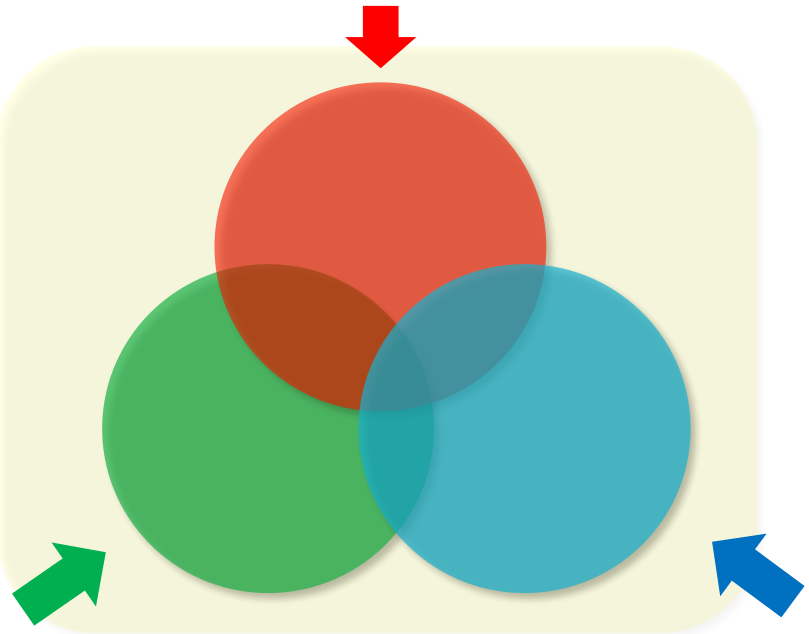
Ken Takahashi, MD, MPH, PHD
Director, Asbestos Diseases Research Institute

- Translational Research
  - From Bench to Bedside
- Australian Situation on Asbestos/ARD
  - Lingering ARD Epidemic
  - Exposure to Asbestos *in situ*
- Translational Research
  - From Bench to Bedside
  - “From Bench to Public”

Asbestos Diseases Research Institute

Laboratory

"understanding the nature and causes of"; "science"



Clinical

"diagnosing and treating"; "treatments"; "medicine"; "health care delivery"

Public Health / Prevention

"preventing"; "preventative" "education and training"; "educational"

The Lab Research Perspective of Asbestos Related Diseases

Dr Yuen Yee Cheng

❑ Laboratory Research Projects

1) *Diagnosis*
   - Develop biomarkers (less-invasive)
   - Discover epigenome to facilitate *Diagnosis*

2) *Disease Mechanism*
   - Identify **microRNAs** with therapeutic potential
   - Artificial **microRNAs** to inhibit growth of MPM
   - Role of YB-1 in drug resistance
   - Mechanisms leading to microRNA dysregulation in MPM

3) *Treatment*
   - 3D cell model of MPM
   - microRNA and drug resistance
   - microRNA regulate PD-L1 expression

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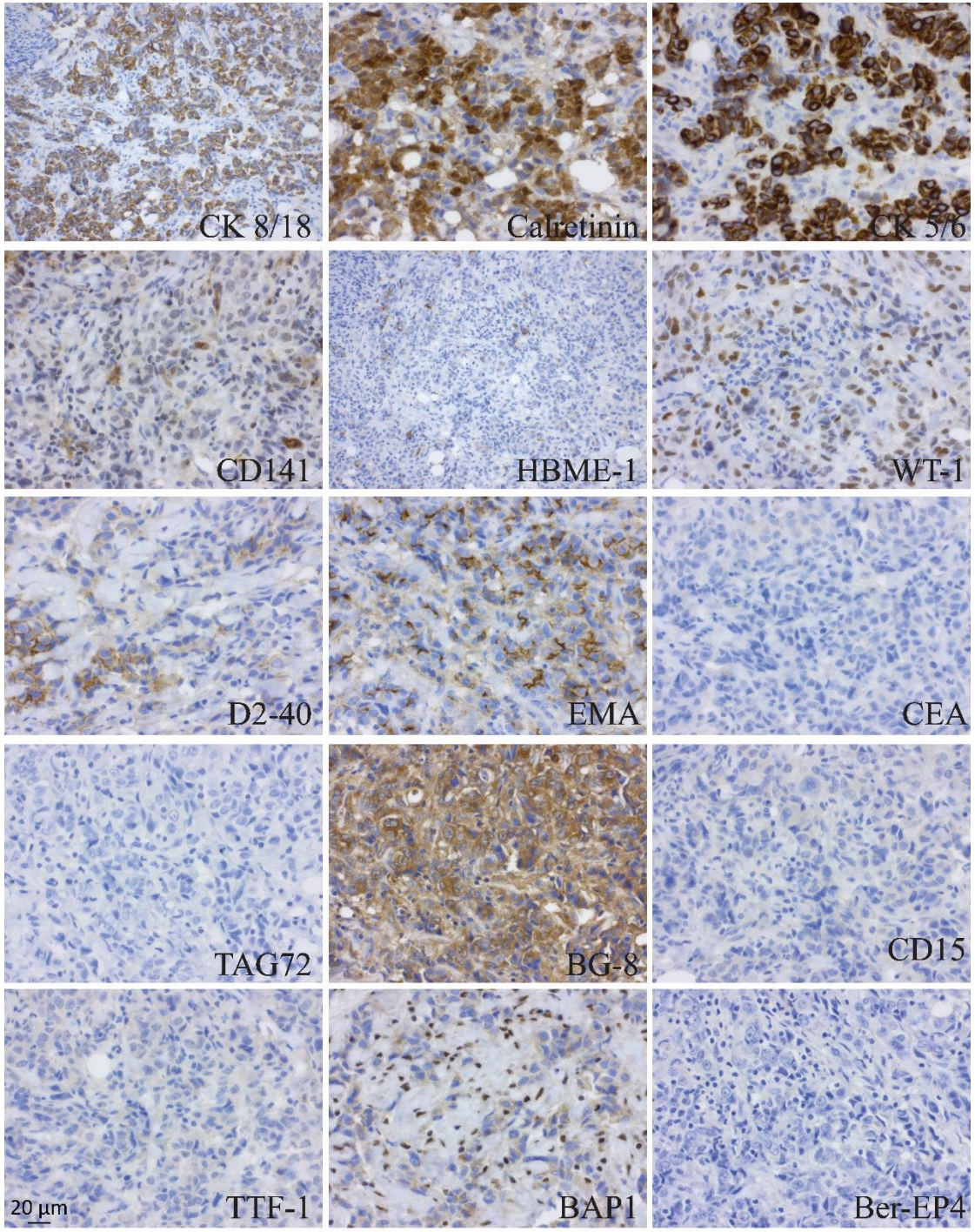
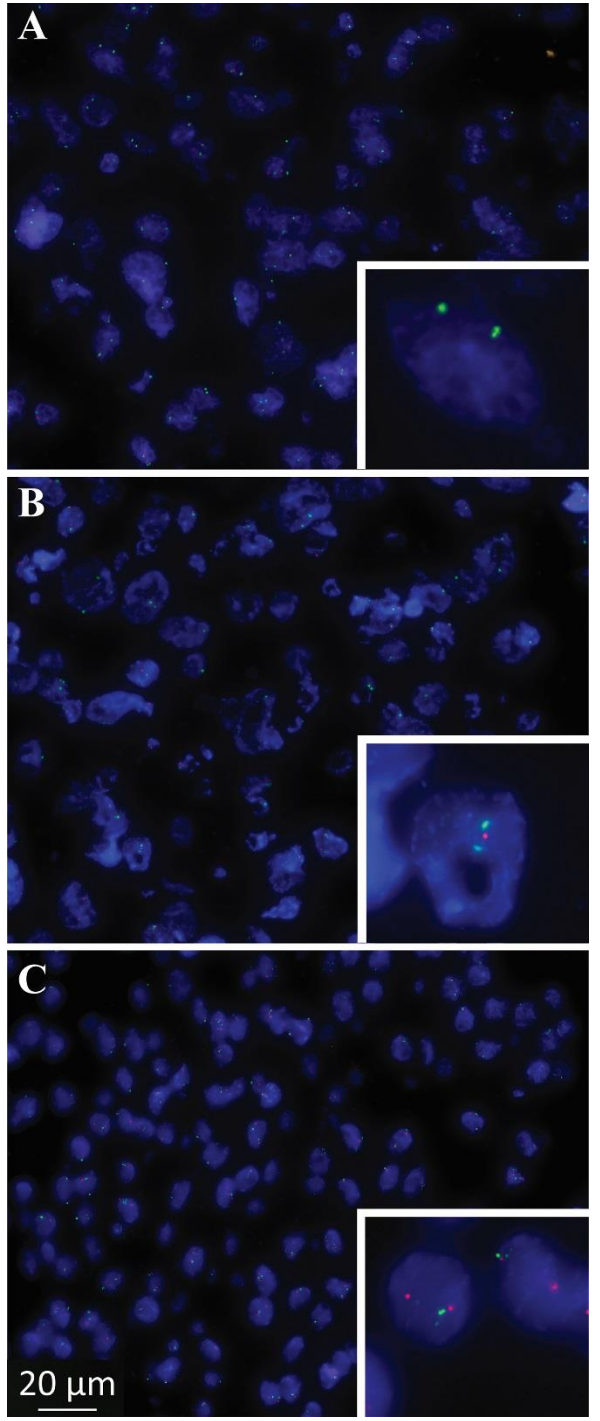
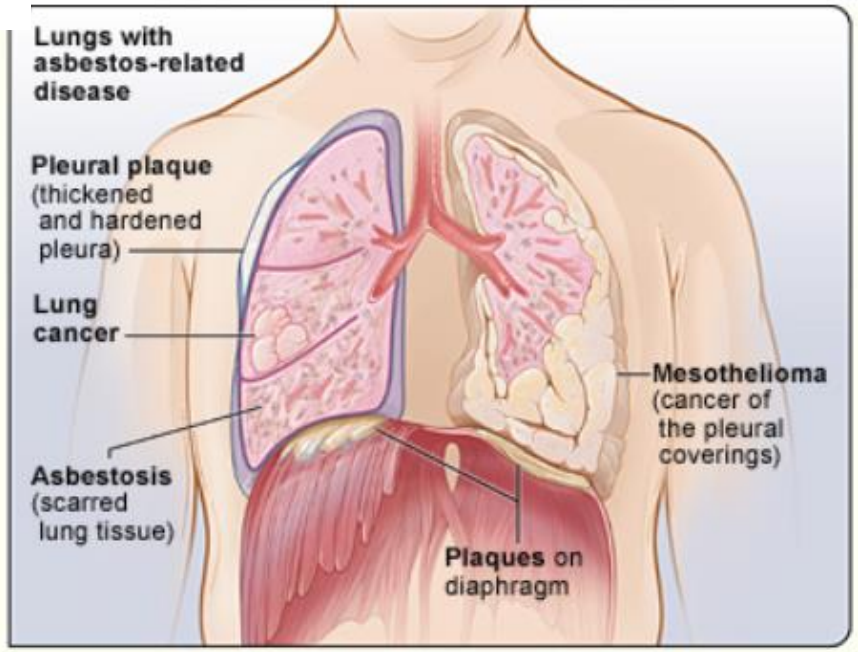
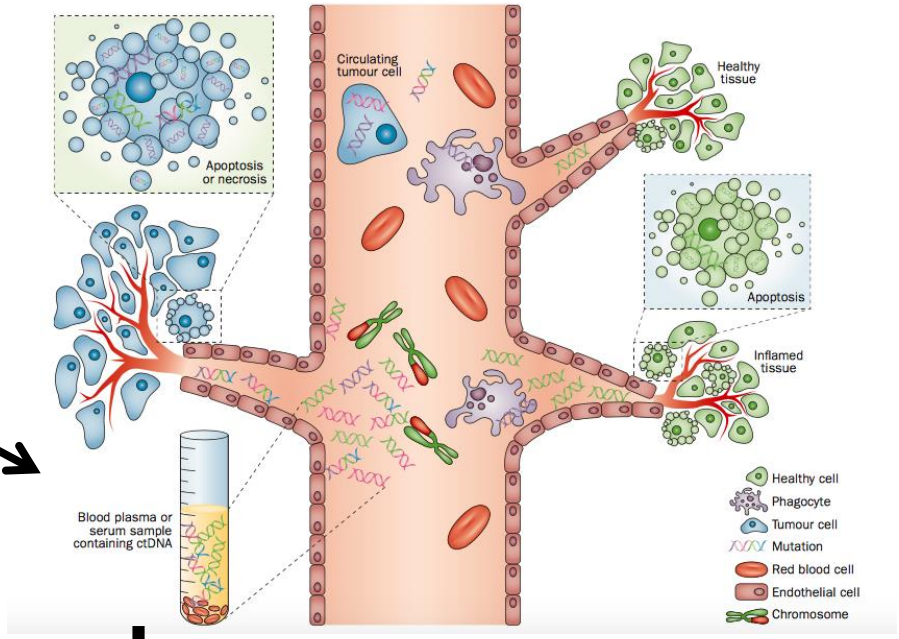




Sarun et al., 2018

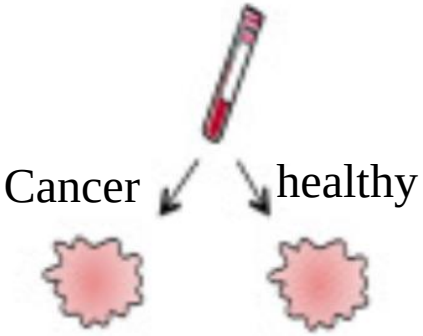




Crowley et al, Nat. Rev. Clin. Oncol 2013

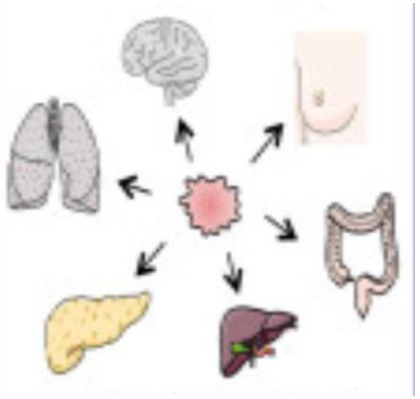
Molecular analysis

Diagnosis of Cancer



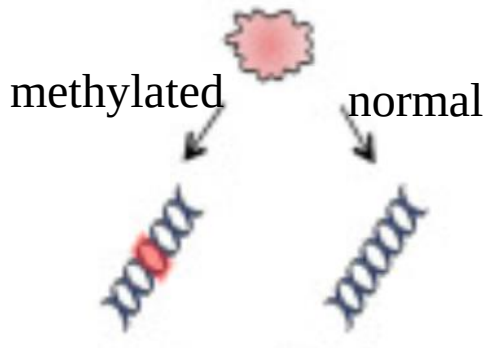
Cancer Dx

Identify tumour type

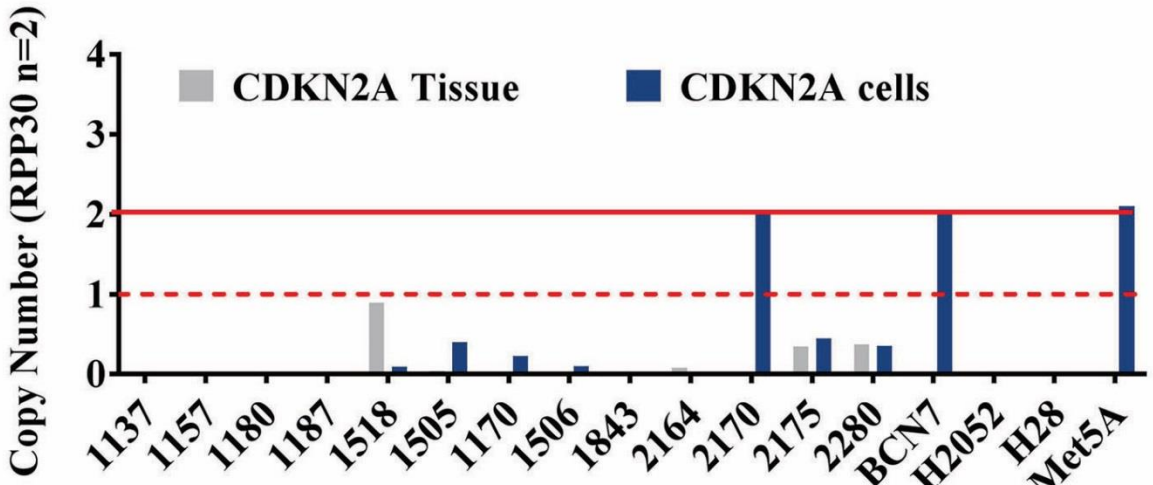


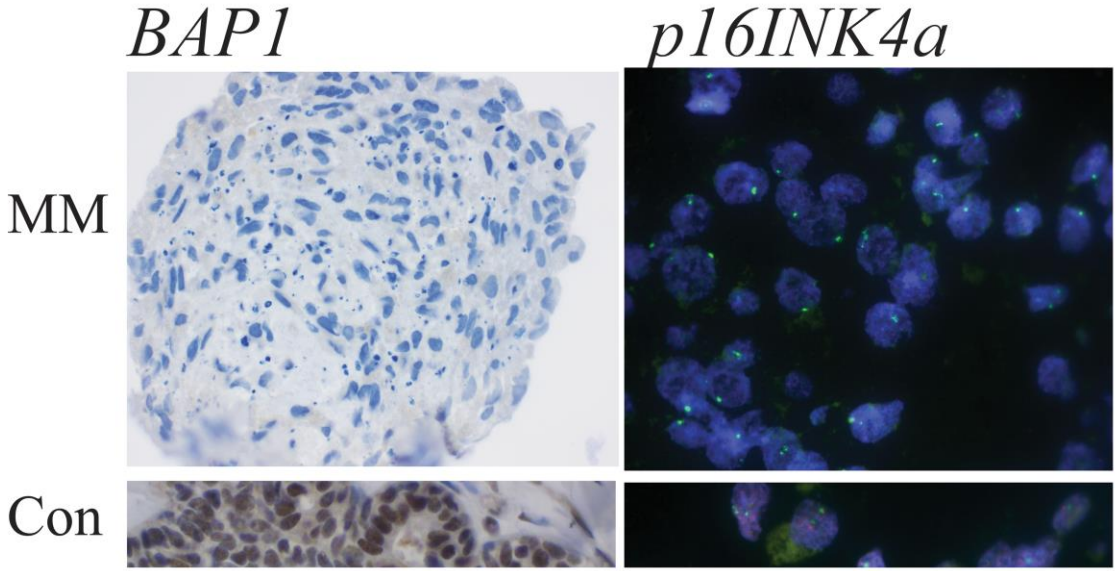
Multi class Cancer Dx

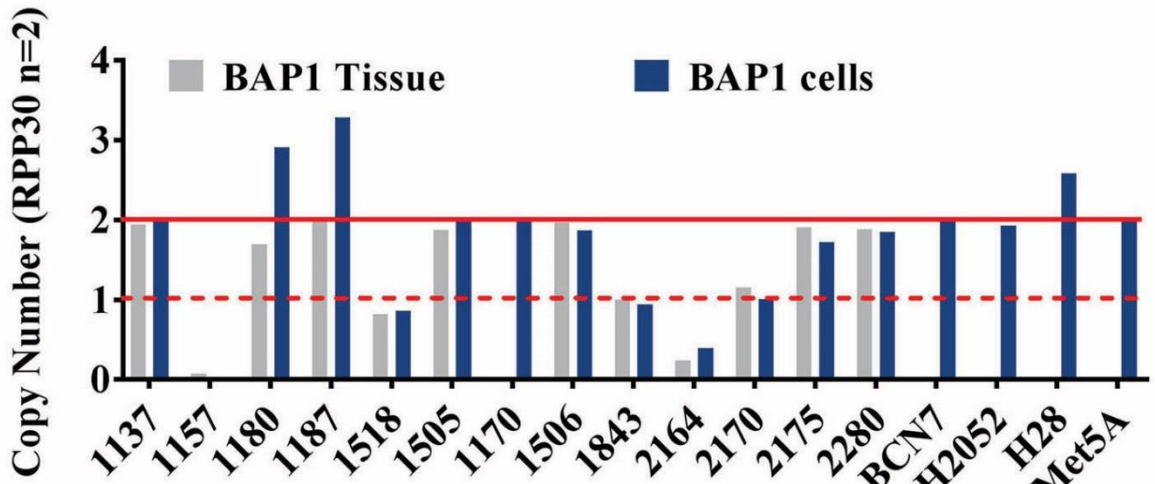
Companion Diagnosis

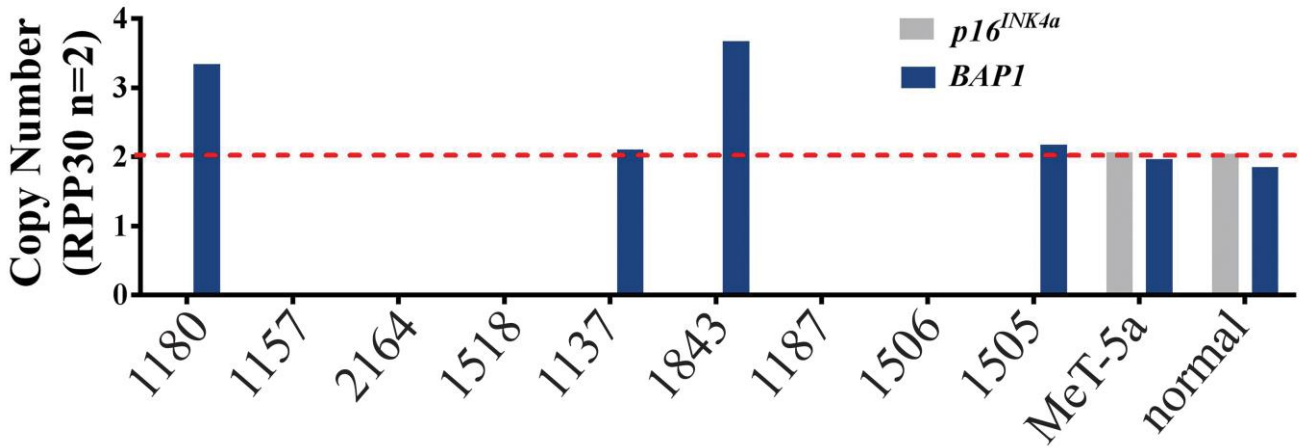


Early cancer biomarker Dx



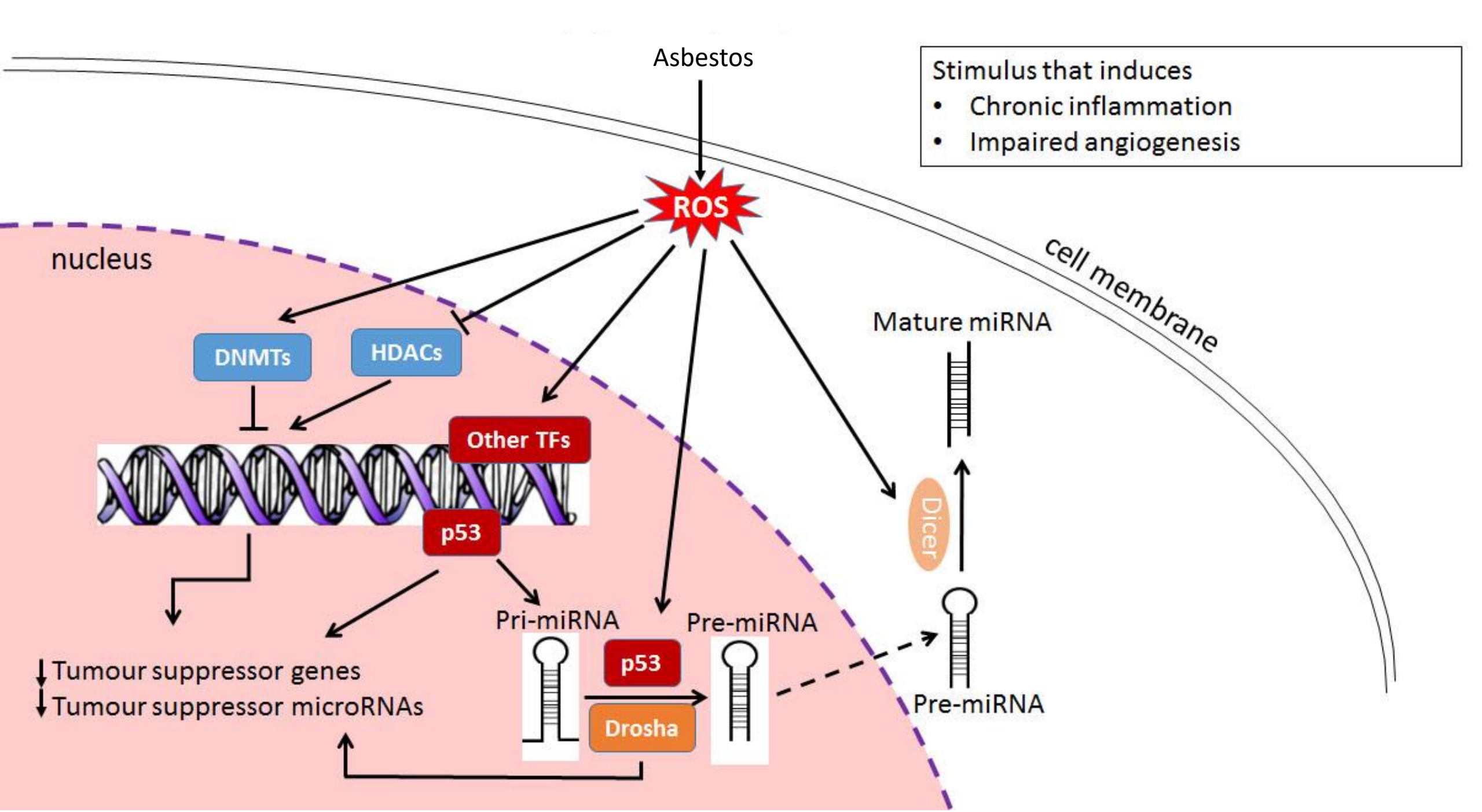






Sarun et al., 2018

Discover epigenome to facilitate *Diagnosis*


Asbestos
Stimulus that induces
• Chronic inflammation
• Impaired angiogenesis
ROS
cell membrane
nucleus
DNMTs
HDACs
Other TFs
p53
Mature miRNA
Dicer
Pri-miRNA
Pre-miRNA
p53
Drosha
Pre-miRNA
Tumour suppressor genes
Tumour suppressor microRNAs

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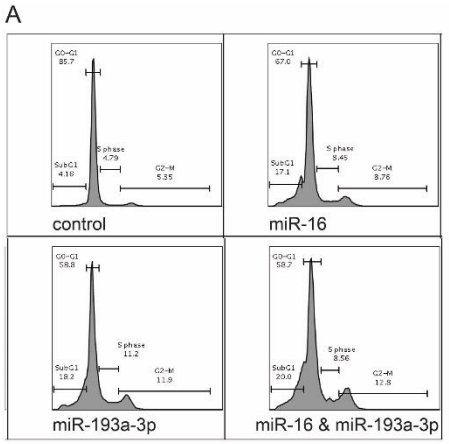
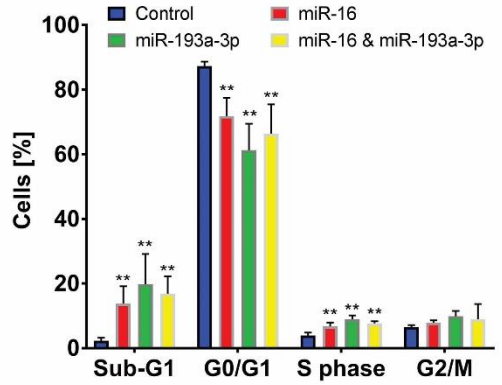
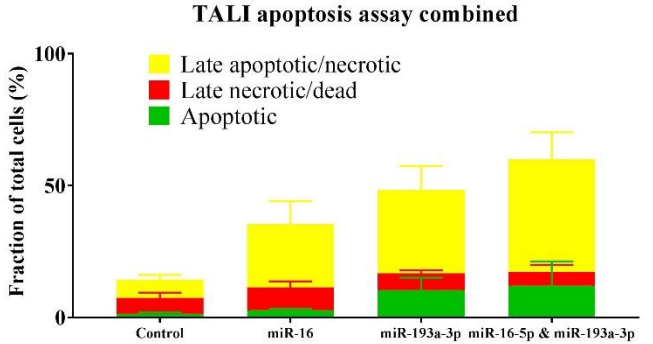
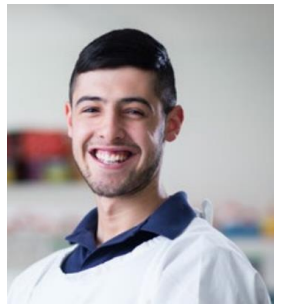
3) *Treatment*
   - 3D cell model of MPM
   - microRNA and drug resistance
   - microRNA regulate PD-L1 expression

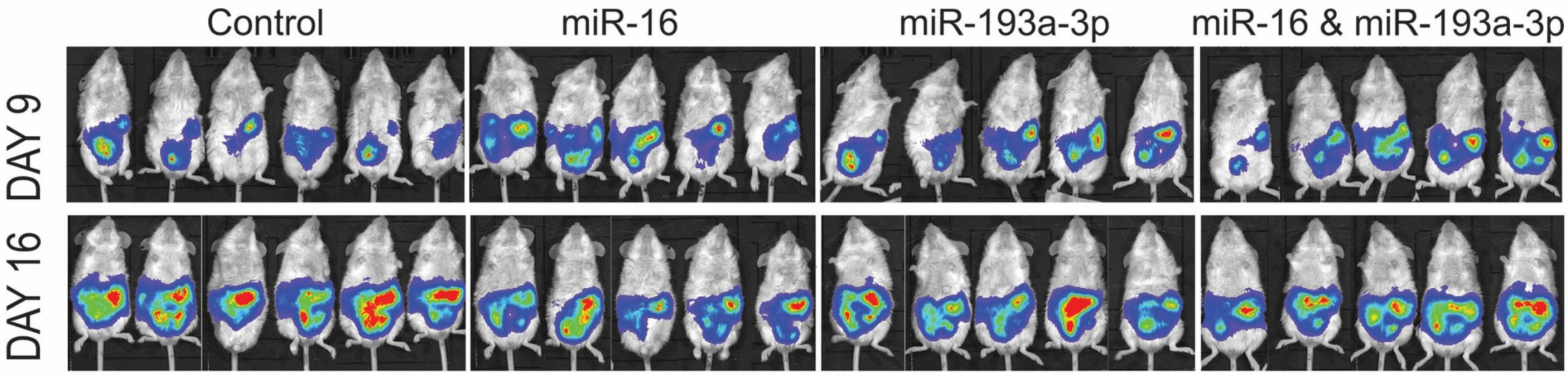
Identify microRNAs with Therapeutic potential



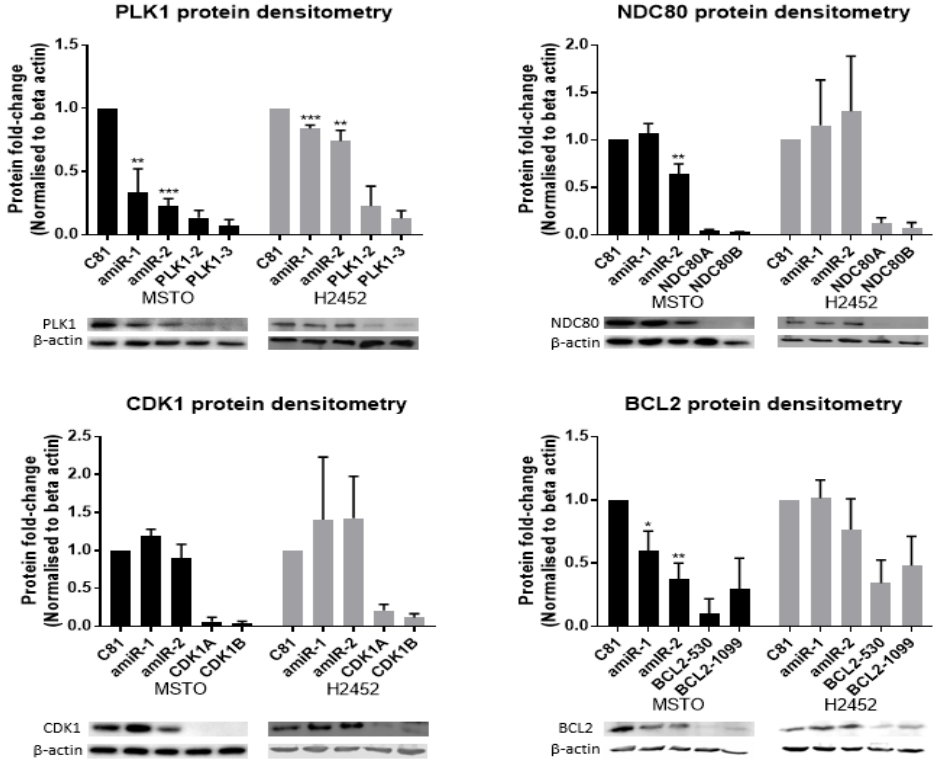


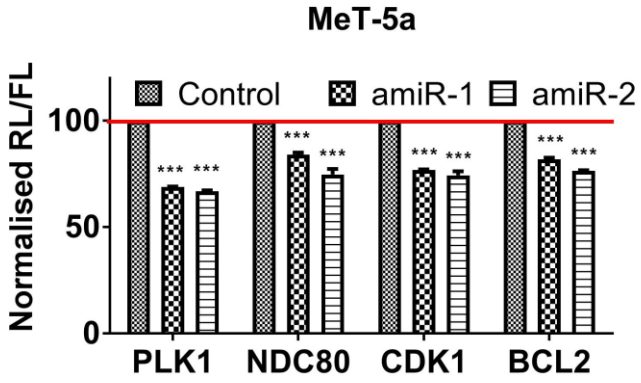


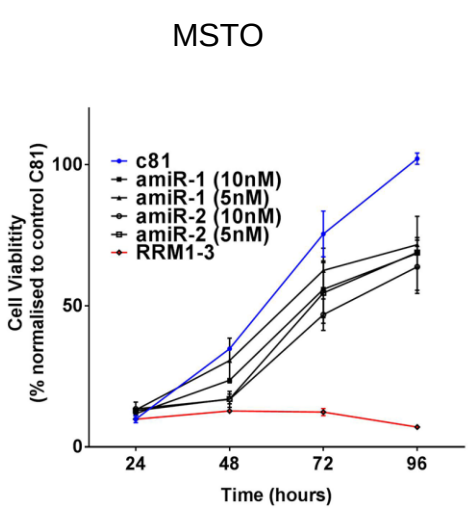


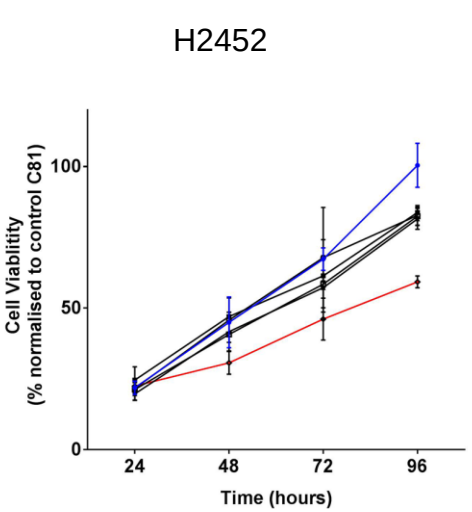


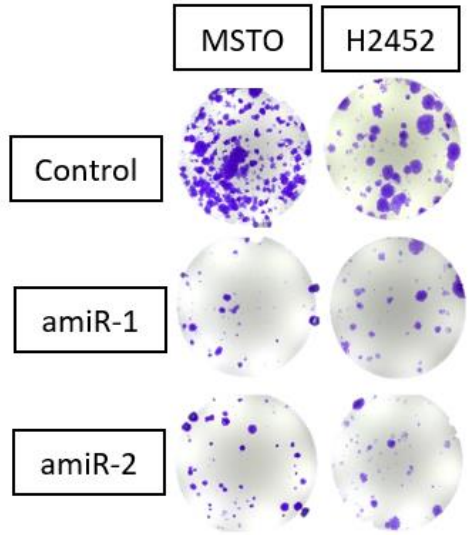
Artificial microRNA

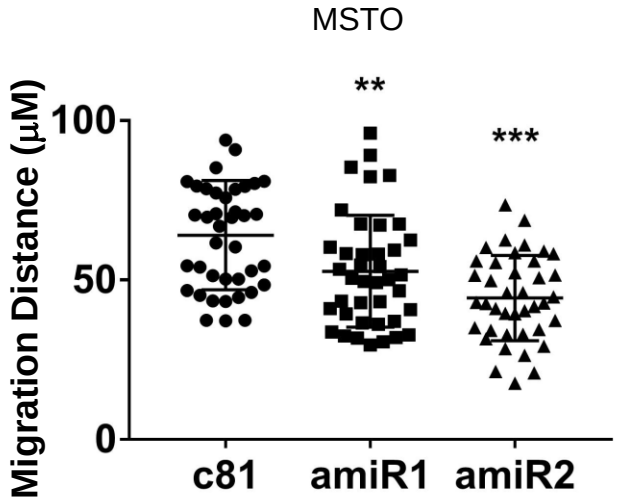


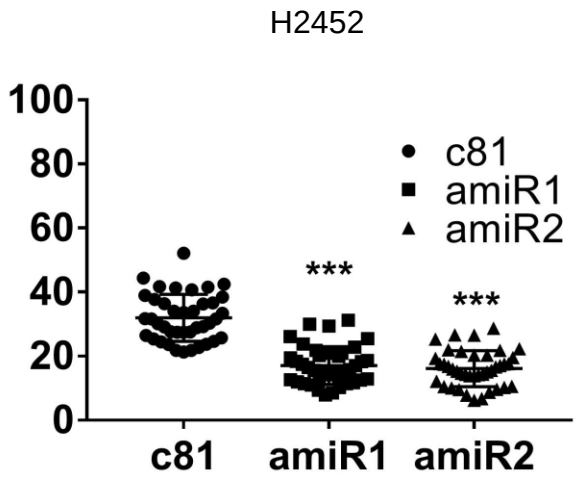








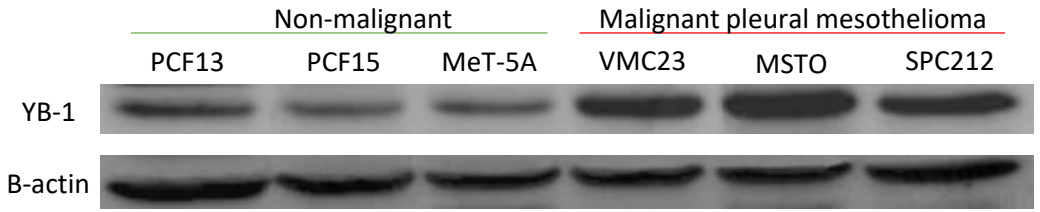




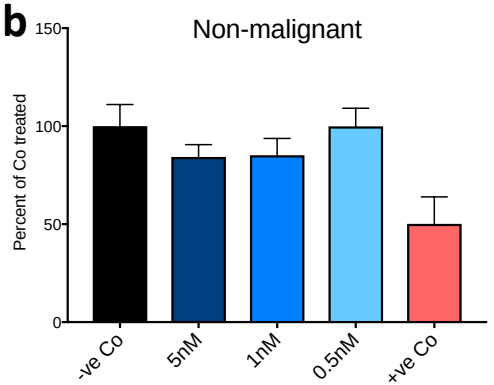
YB-1: a novel therapeutic target in mesothelioma?

Thomas Johnson (PhD candidate)

1a

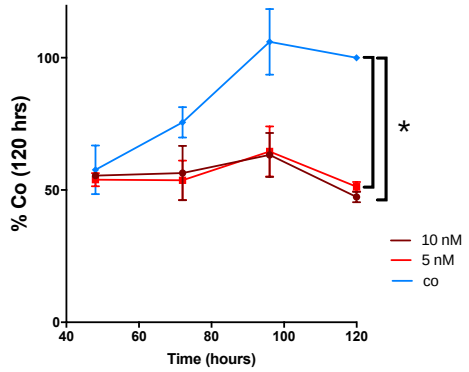


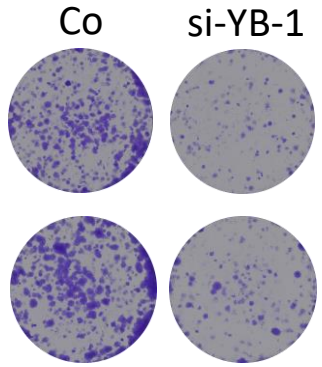
1b



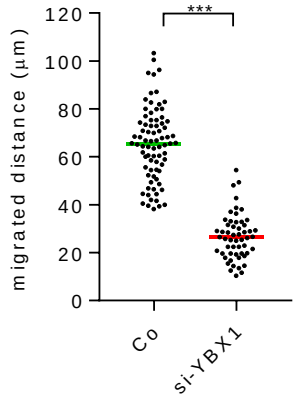
(a) YB-1 is overexpressed in mesothelioma and (b) knockdown doesn't affect non-malignant cell growth

2a

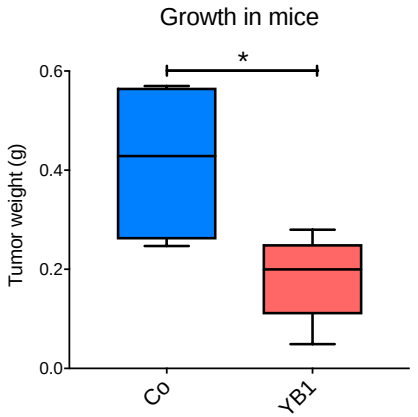


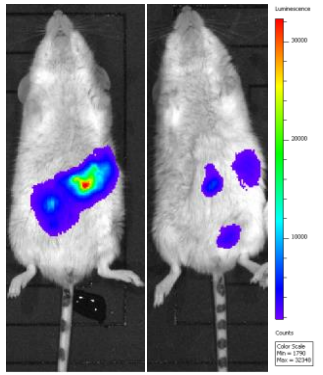


2b



(a) YB-1 knockdown inhibits mesothelioma growth and (b) migration *in vitro*

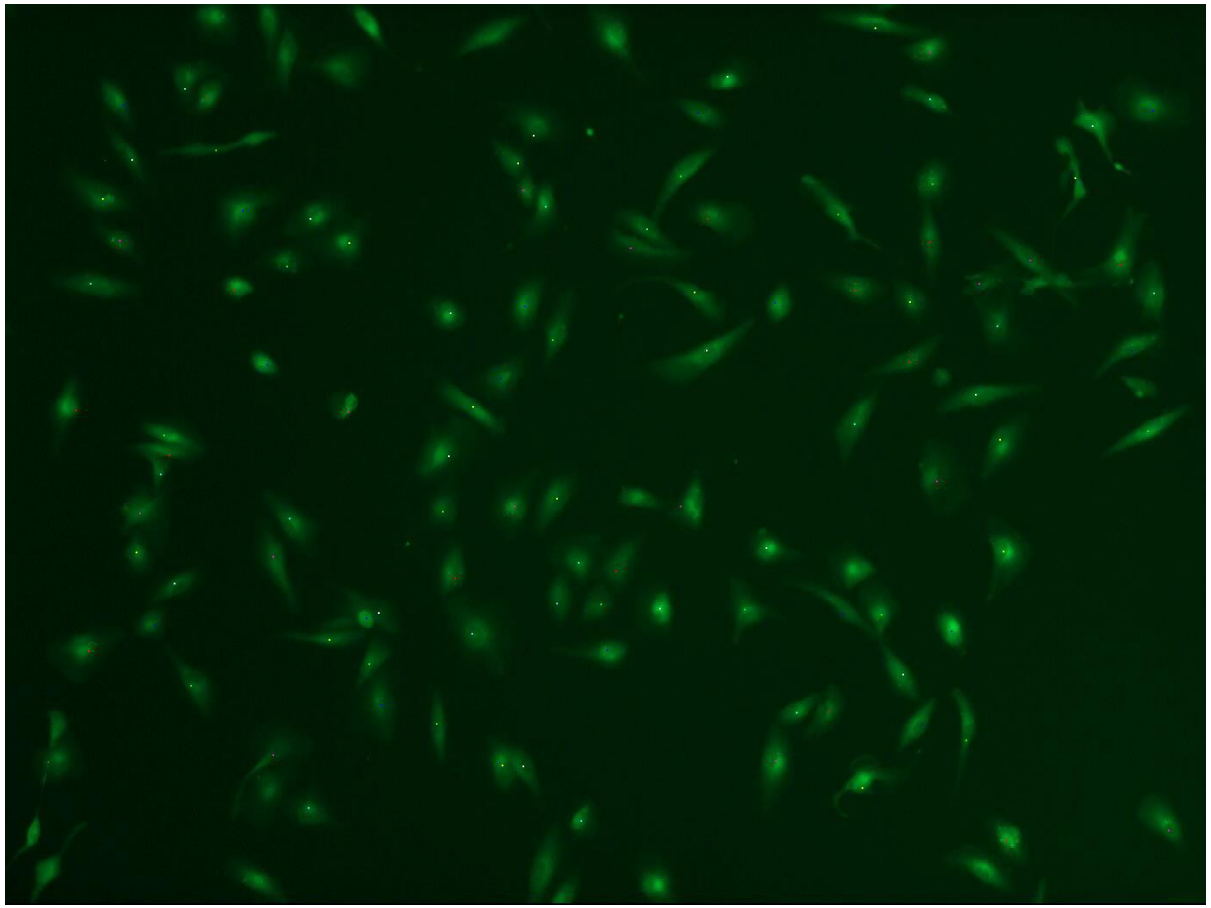




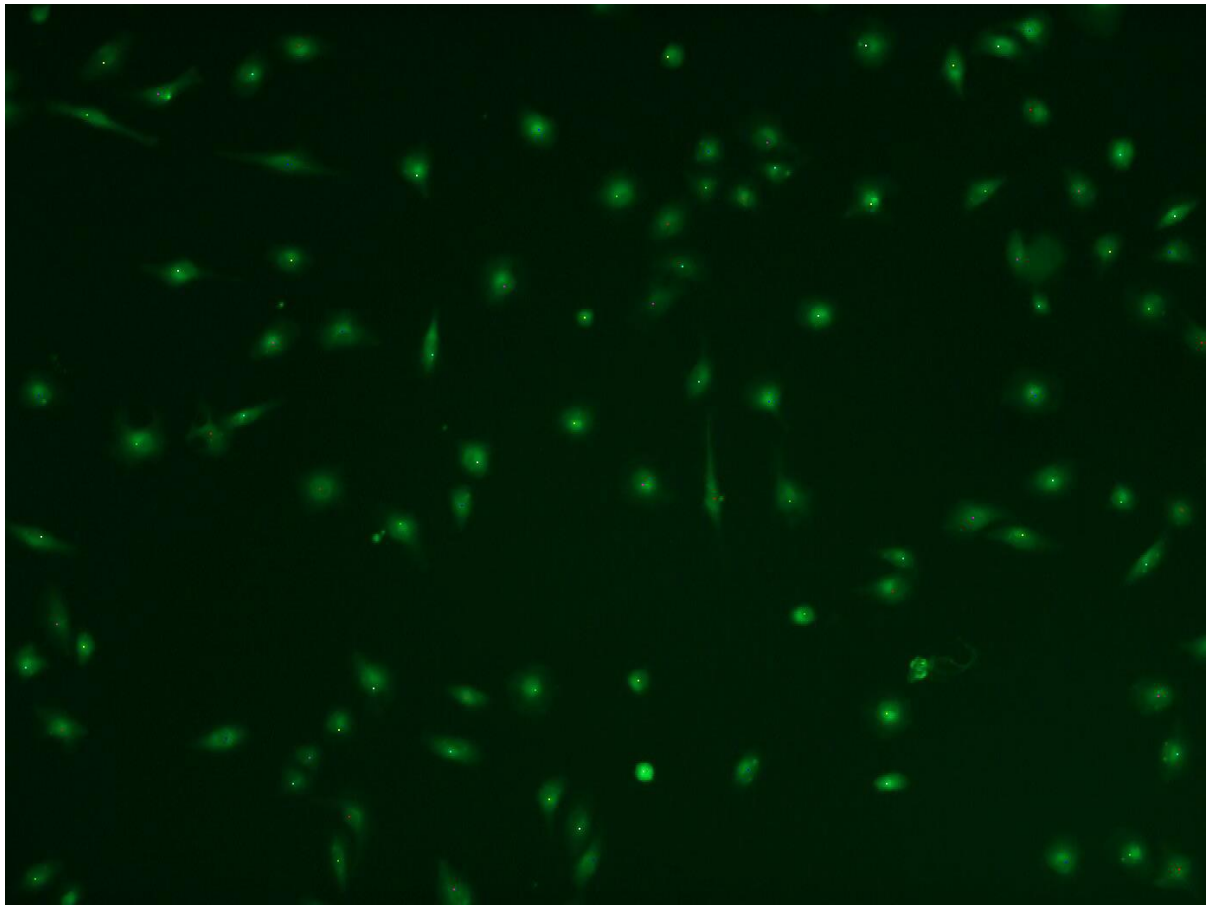
YB-1 knockdown inhibits human mesothelioma growth in mice

Video migration analysis of mesothelioma cells

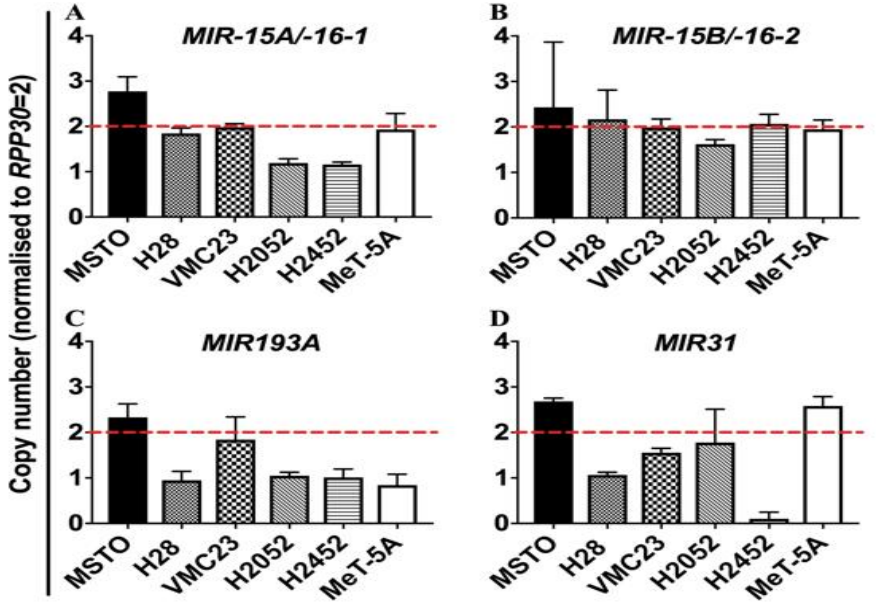
Control treated



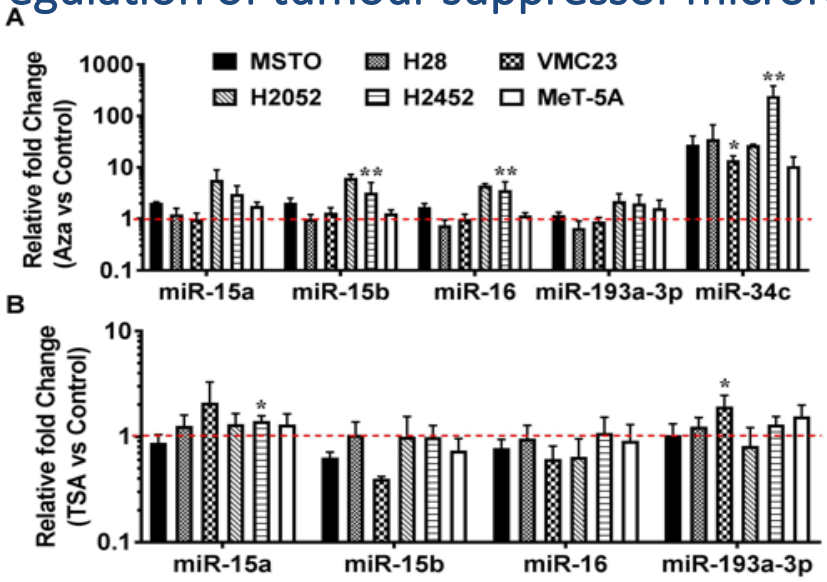
YB-1 inhibited



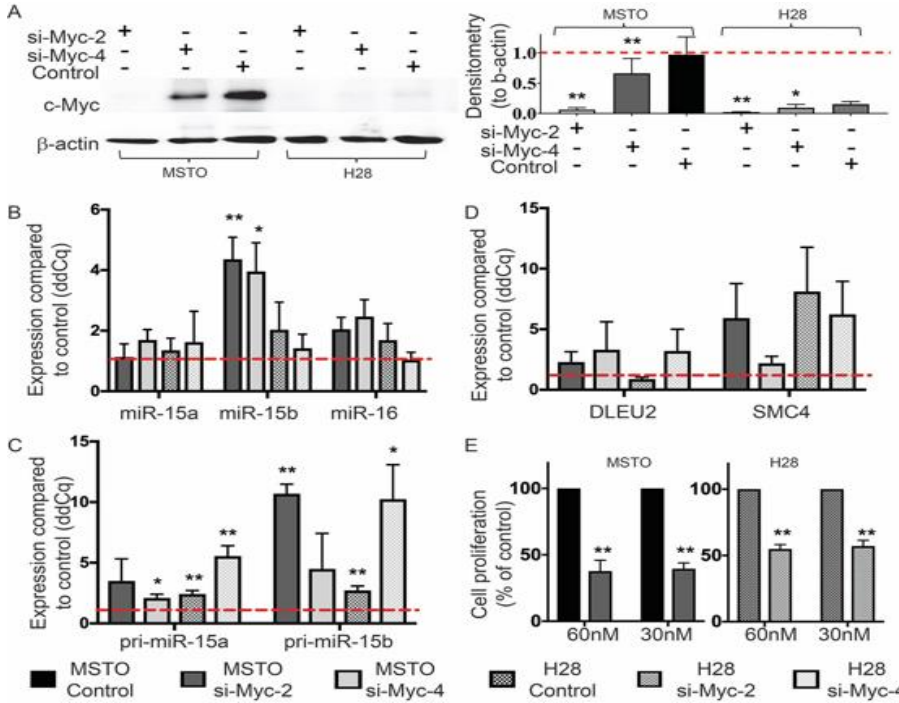
Multiple mechanisms contribute to the downregulation of tumour suppressor microRNAs in MPM



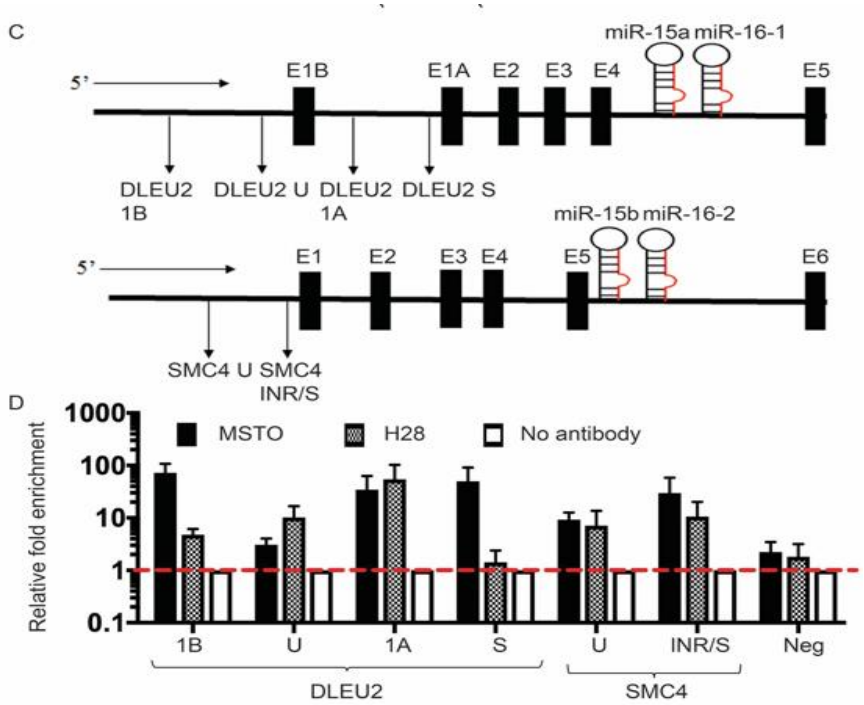
Genomic deletion leads to the loss of miR-193a-3p as shown by copy number loss in the majority of cell lines



Epigenetic mechanisms are not large contributors to microRNA loss in MPM cell lines



C-Myc knockdown leads to the transcriptional up-regulation of miR-15/16- most predominately via the miR-15b/16-2 locus



C-Myc directly associates with the miR-15a/16-1 and miR-15b/16-2 promoter regions

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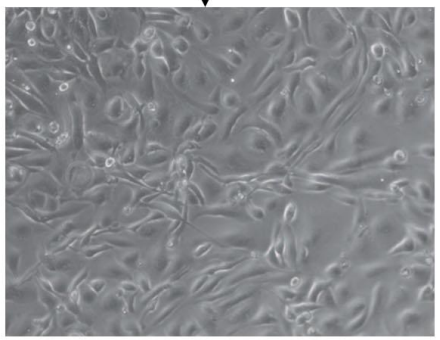
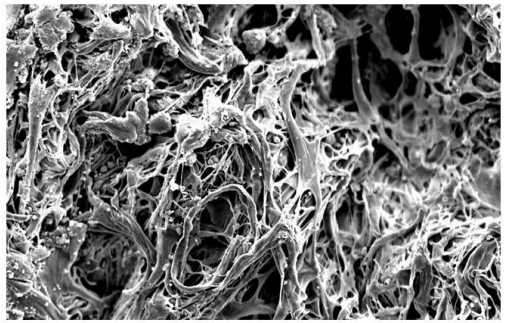
Current cell model in 2D



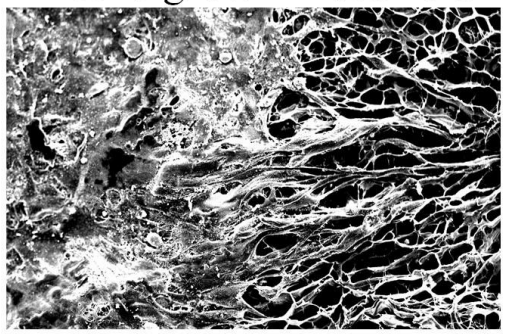
- Monolayer, poor cell-cell signaling
- Lack of extracellular matrix
- Lack of hypoxia

→ not similar to true biology

Scaffold (SEM)

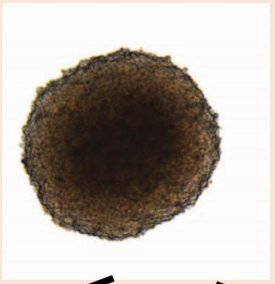


3D cells grown in scaffold



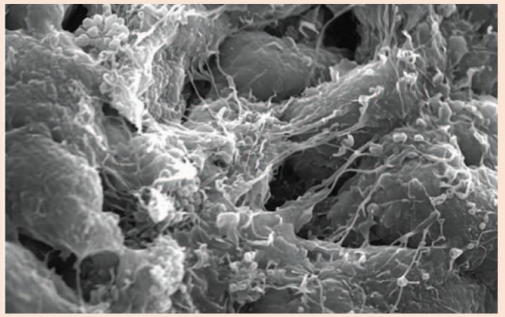
- Multilayer, some cell-cell signalling
- Unnatural extracellular matrix
- Lack of hypoxia

→ Not similar to "true" biology

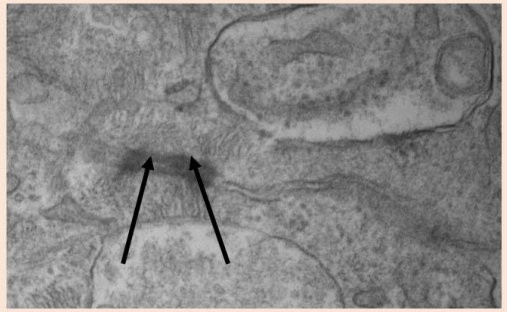


- 3D spheroids,
- without scaffold,
- with microgravity

SEM



TEM

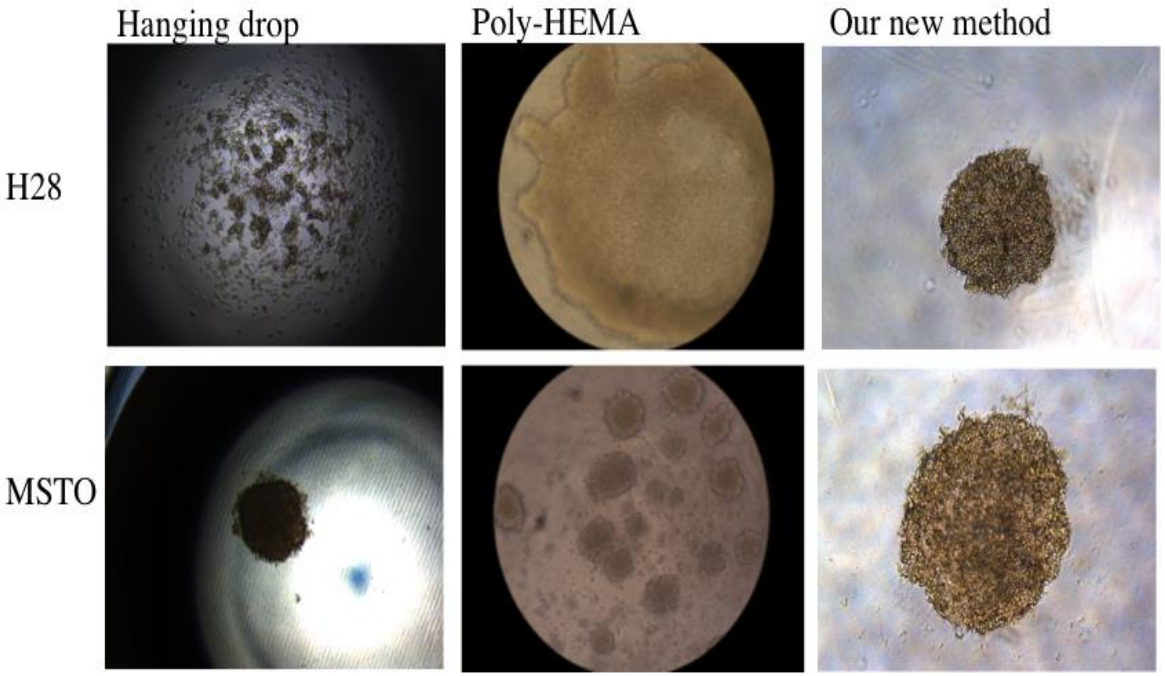


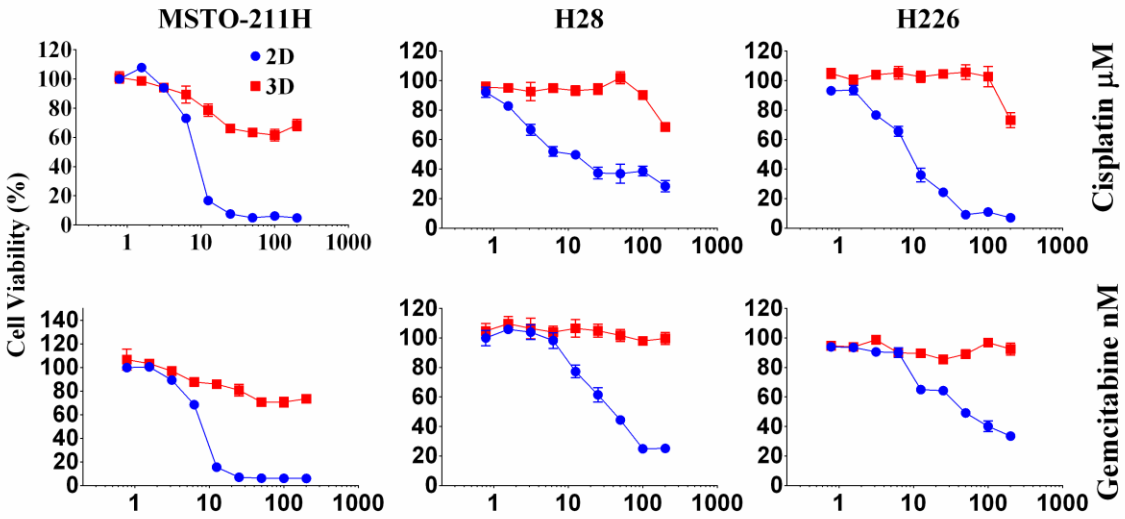
- Multilayer, realistic cell-cell signalling
- Naturally occurring extracellular matrix
- Realistic hypoxic core

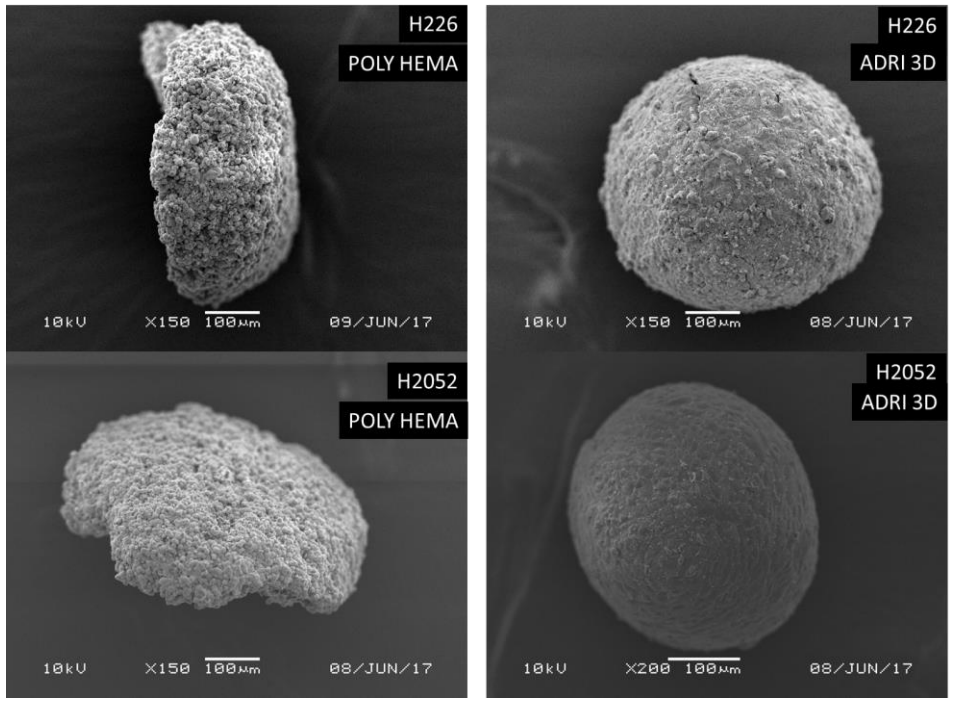
→ **Similar to "true" biology**

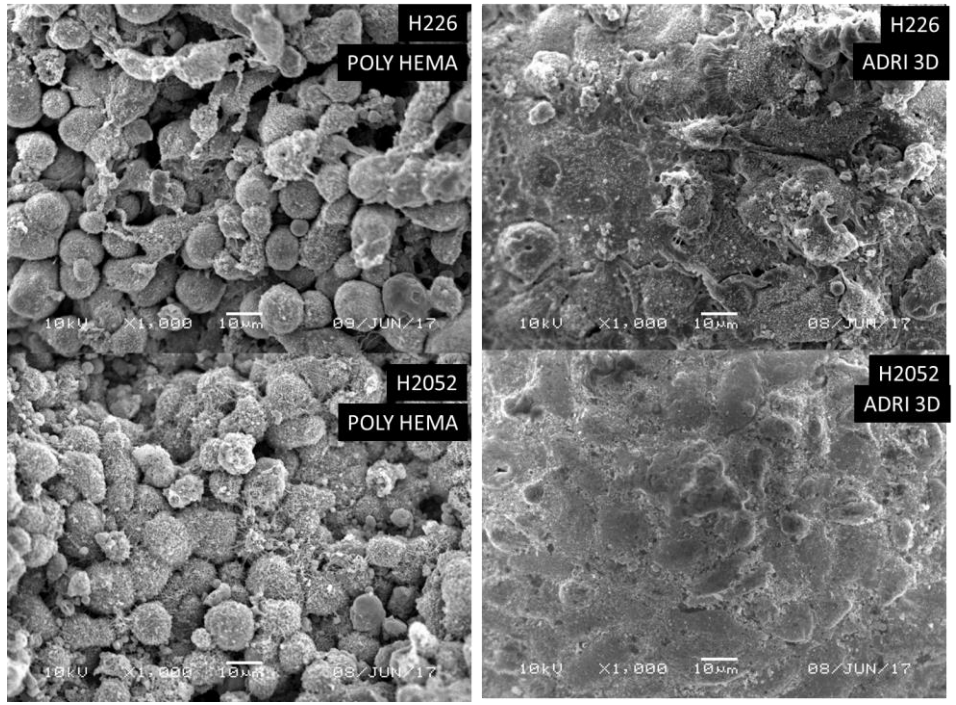
Our Proof-of-concept experiments demonstrated

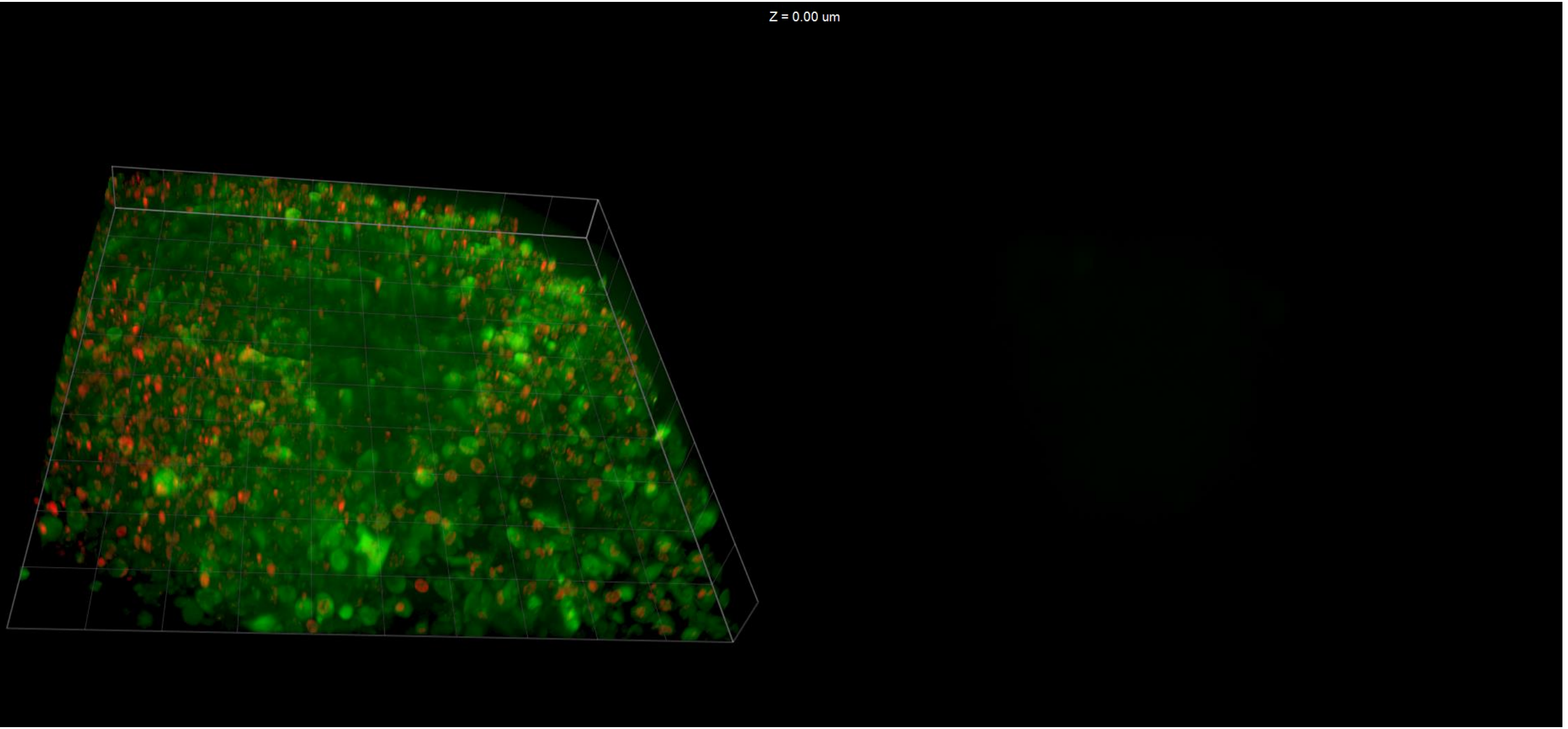
- Healthy cell morphology (see SEM image)
- Realistic tight junctions (see arrows in TEM Image)


Hanging drop
Poly-HEMA
Our new method
H28
MSTO


MSTO-211H
H28
H226
2D
3D
Cell Viability (%)
Cisplatin µM
Gemcitabine nM

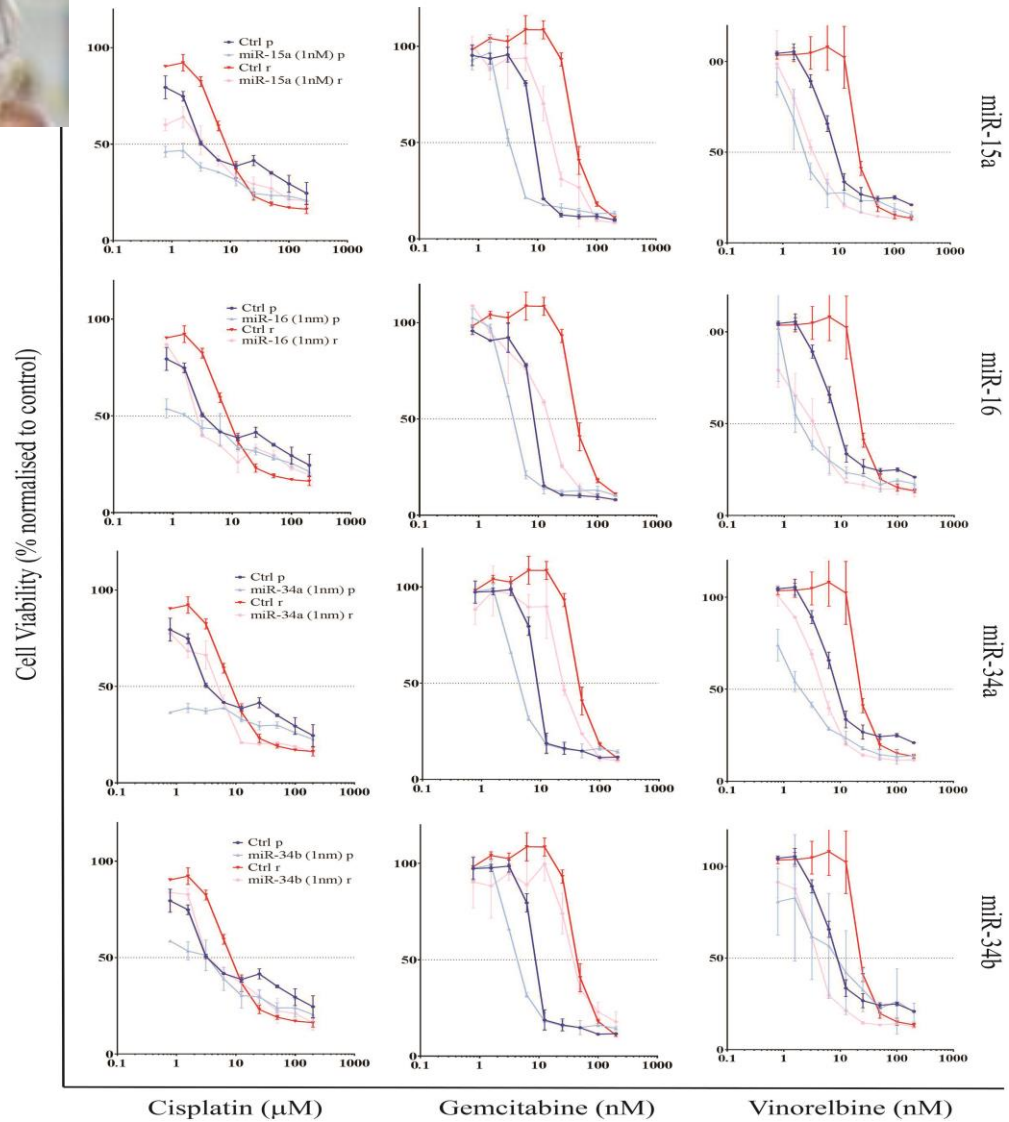

H226
POLY HEMA
H226
ADRI 3D
H2052
POLY HEMA
H2052
ADRI 3D

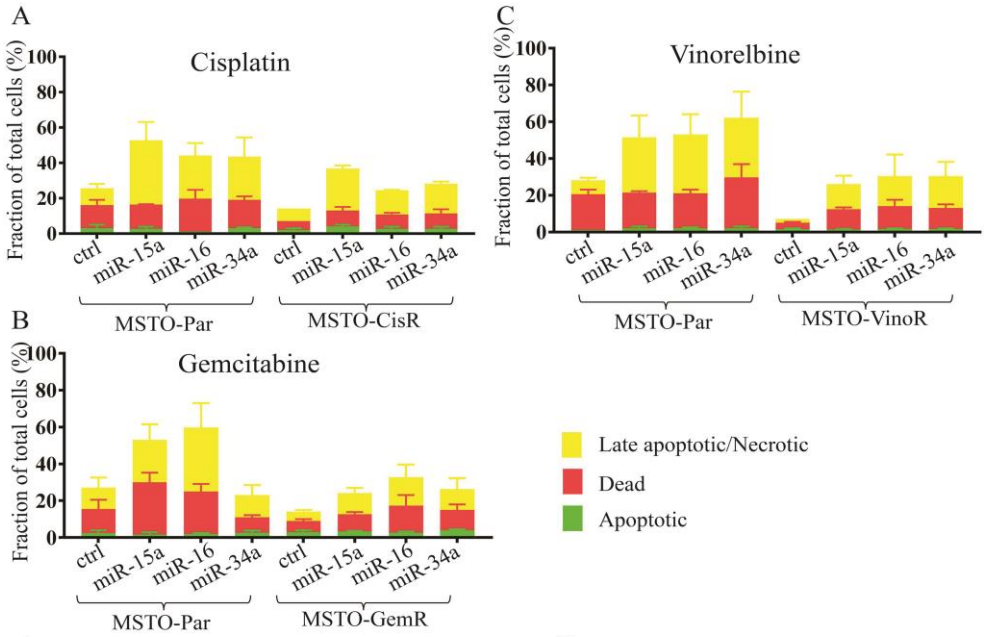

H226
POLY HEMA
H226
ADRI 3D
H2052
POLY HEMA
H2052
ADRI 3D

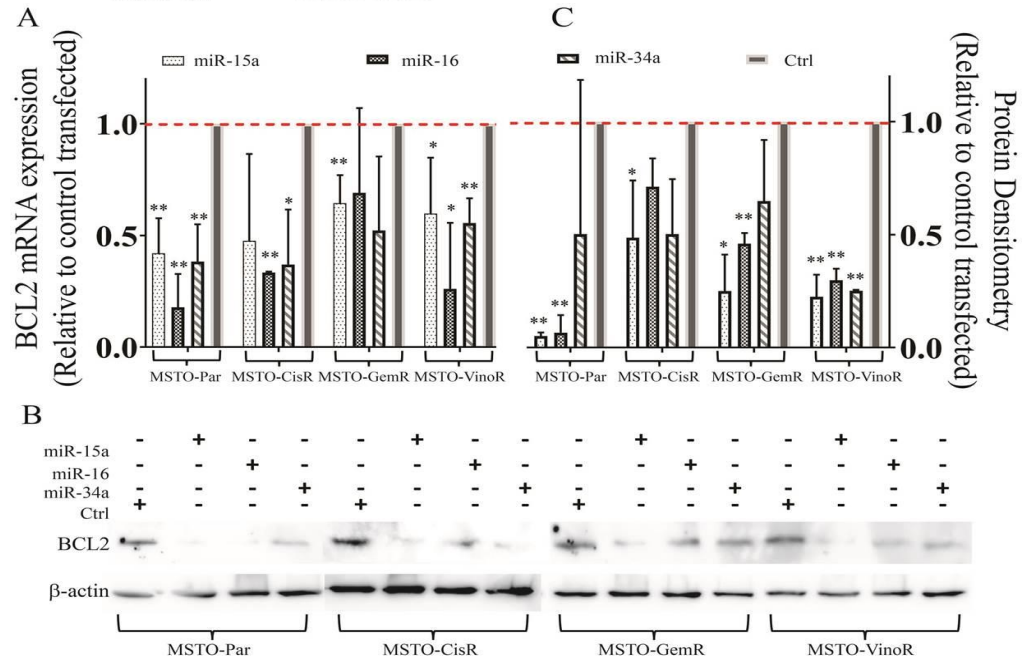

Z = 0.00 um

Tumour suppressor microRNAs sensitise MPM drug resistant cell lines to chemotherapy

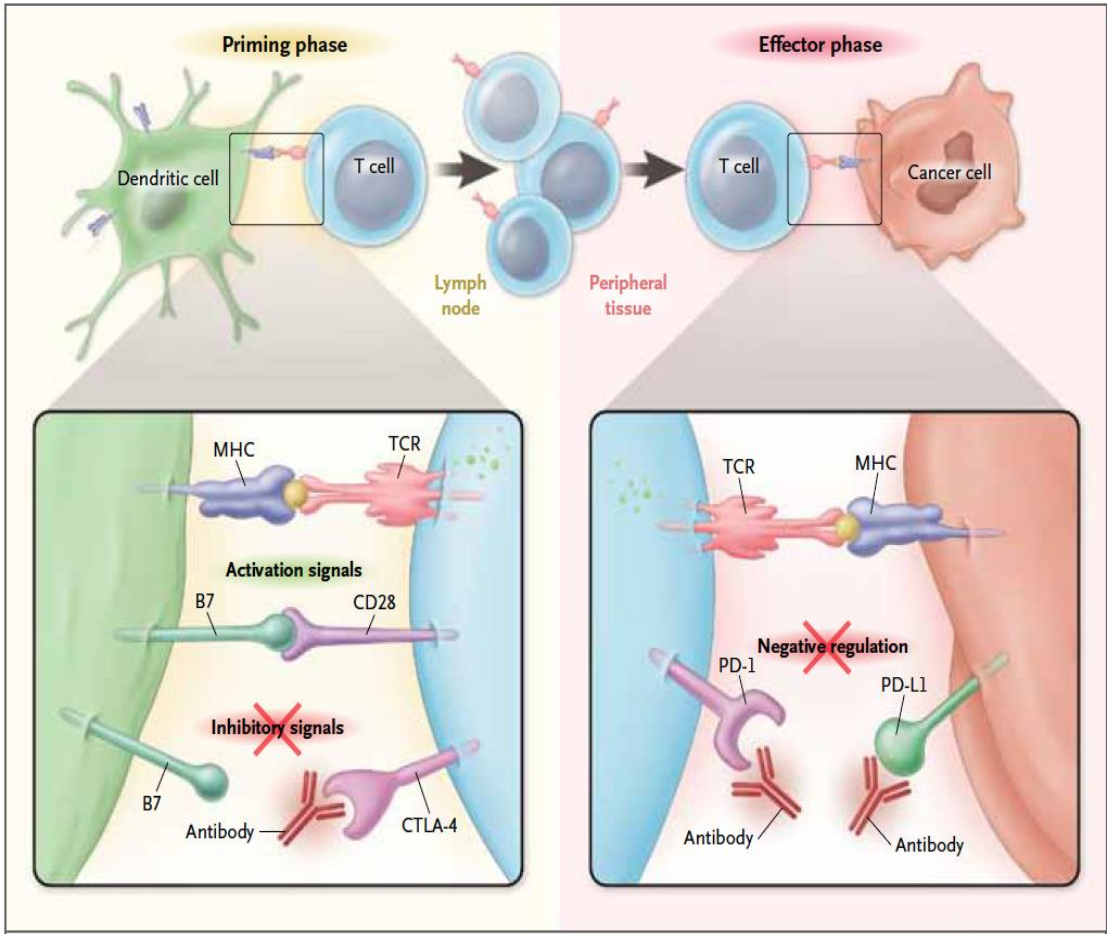
microRNA restoration increases **chemotherapy sensitivity** in MPM cells via **regulation of apoptosis**



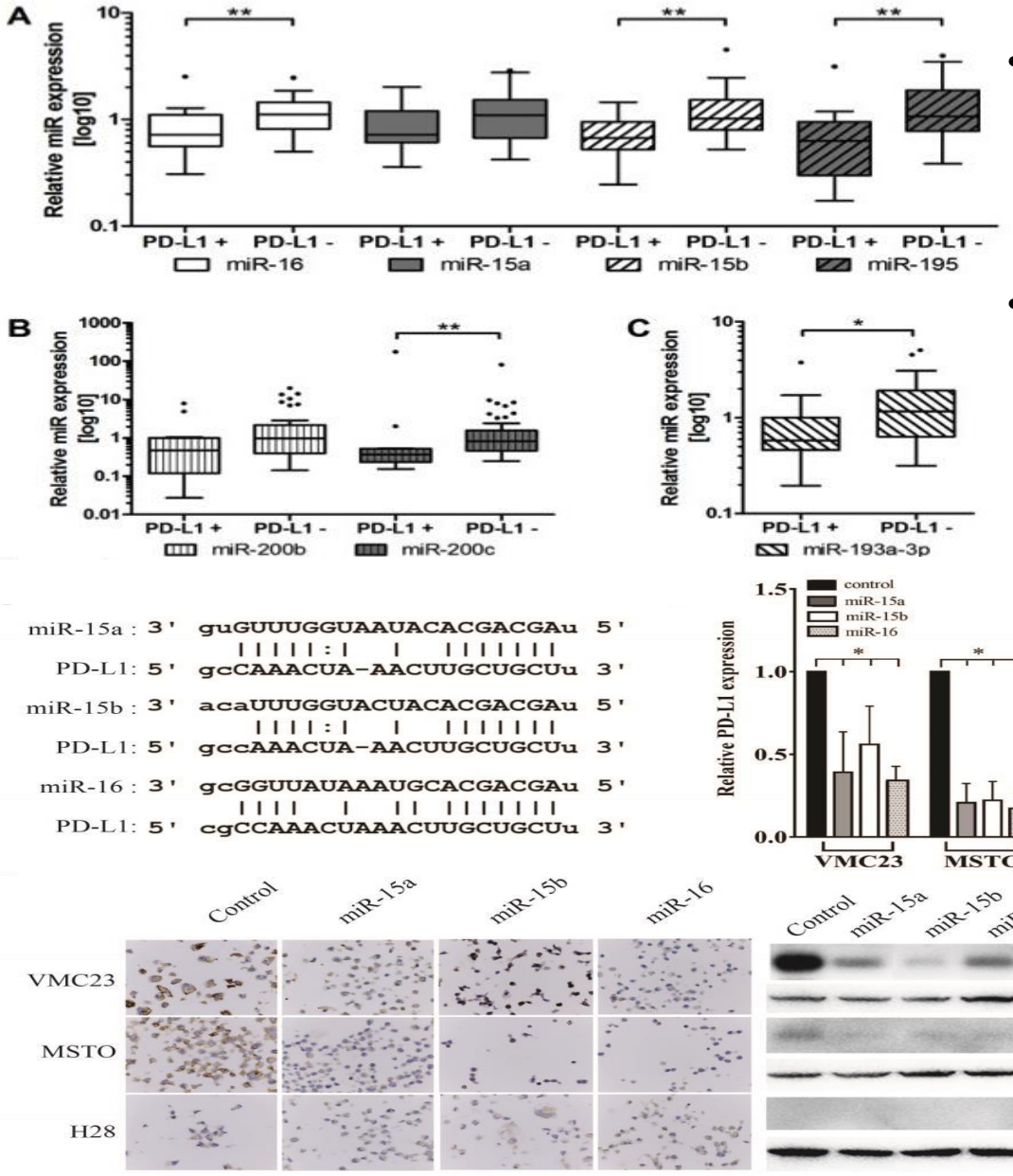




Tumour suppressor microRNAs attenuate the level of immune checkpoint molecule PD-L1 and sensitise to chemotherapy in MPM- potential combination therapy in MPM



Antibodies can block the anti-immune response of the **PD-1/PD-L1 axis** in tumours



- **microRNA** levels are **lower** in **PD-L1 positive** MPM tumours
- **miR-15/16** restoration **reduces PD-L1** levels

Future directions

- Improve MPM diagnosis by further validating candidate biomarkers by utilising biospecimen's in the ADRI biobank

- Improve MPM treatment by testing microRNA based *in vitro* findings in preclinical models

- Improve the outcomes of current treatment options in MPM using a combinatorial approach with immunotherapy

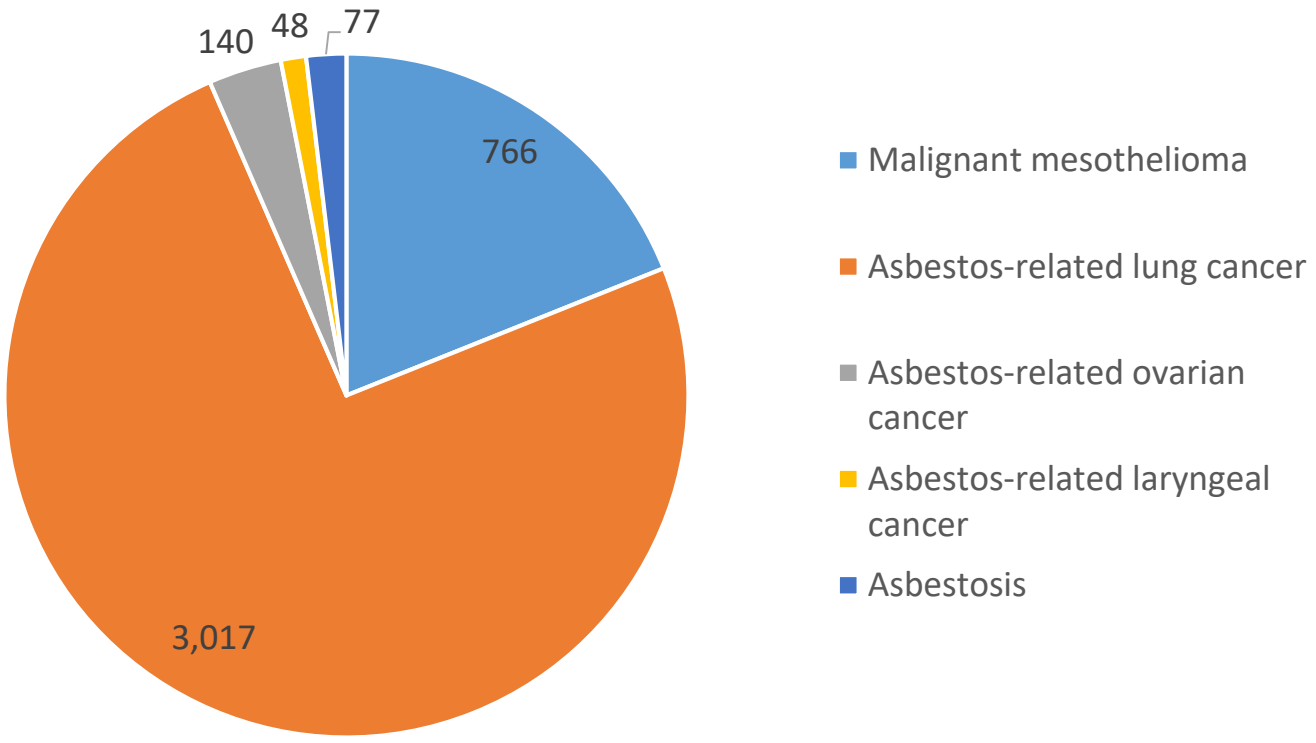
Towards a better understanding of asbestos-related disease

Dr Matthew Soeberg

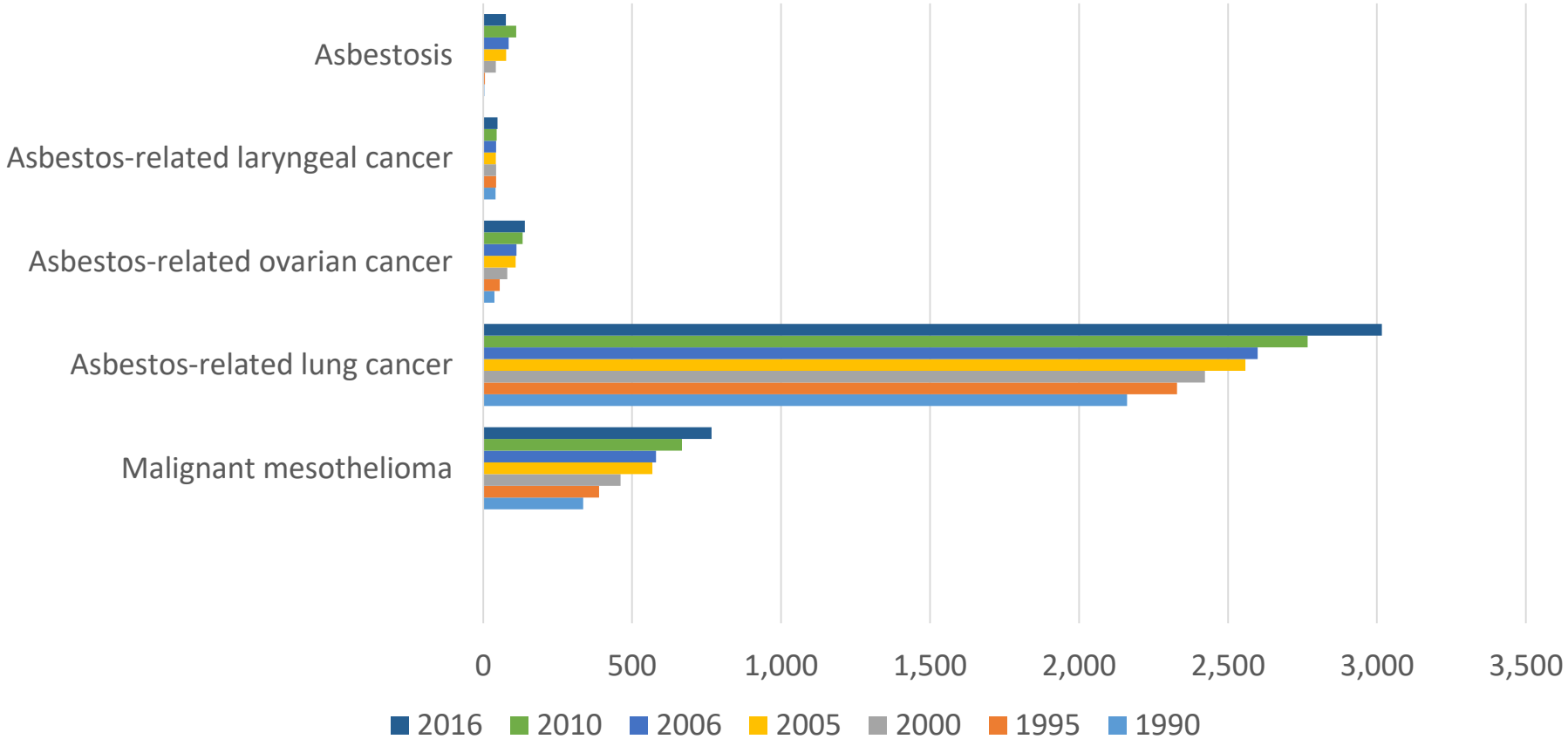
A broader view of asbestos-related disease

- Asbestosis
- Asbestos-related lung cancer
- Laryngeal cancer
- Ovarian cancer
- Malignant mesothelioma – data most often presented
- Pleural plaques

What do the global burden of disease data tell us about asbestos-related disease from occupational exposure in Australia in 2016?

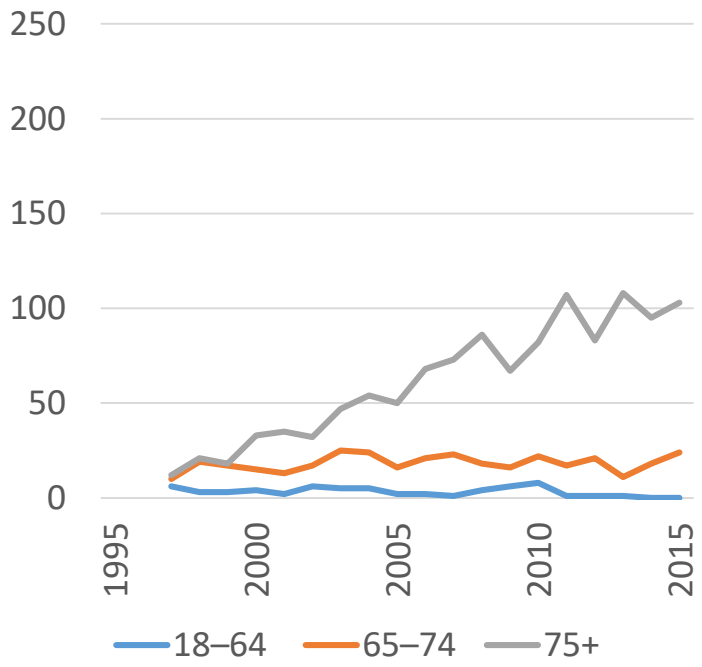


What do the global burden of disease data tell us about asbestos-related disease from occupational exposure in Australia over time?

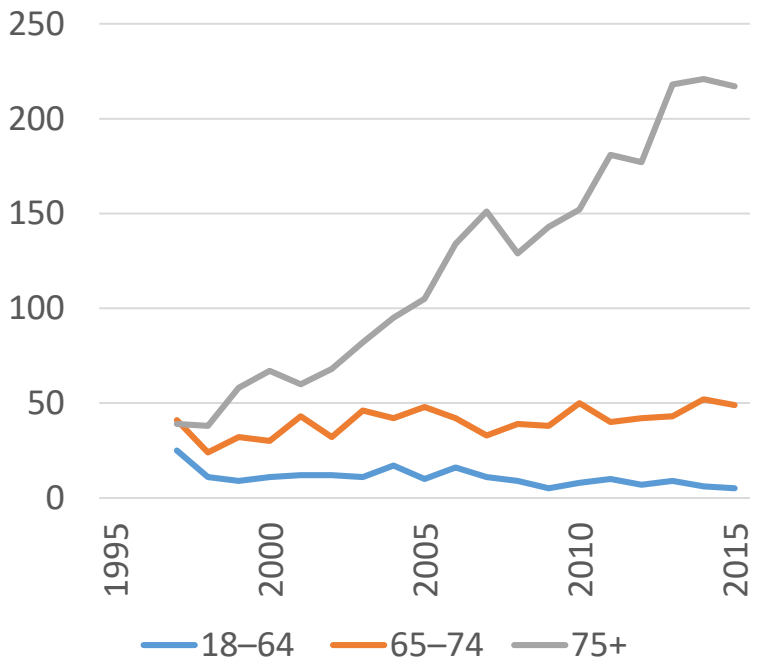


What can other data from Australia tell us? Asbestosis as a cause of death

Asbestosis as the primary cause of death – by age group

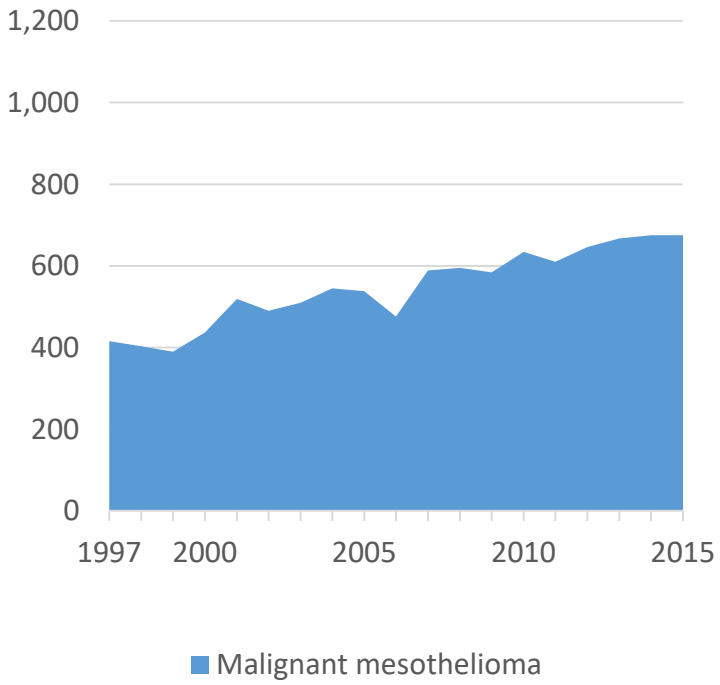


Asbestos as a secondary cause of death – by age group

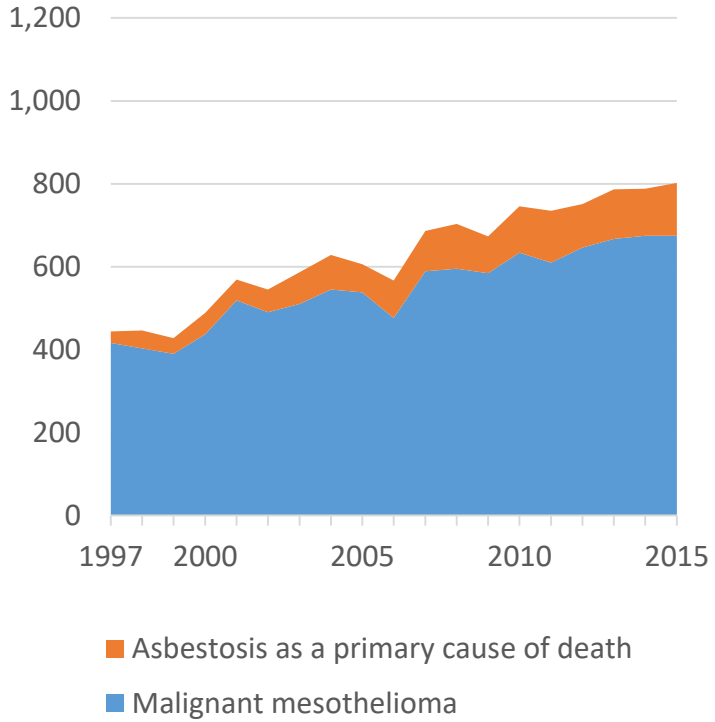


What can other data from Australia tell us?

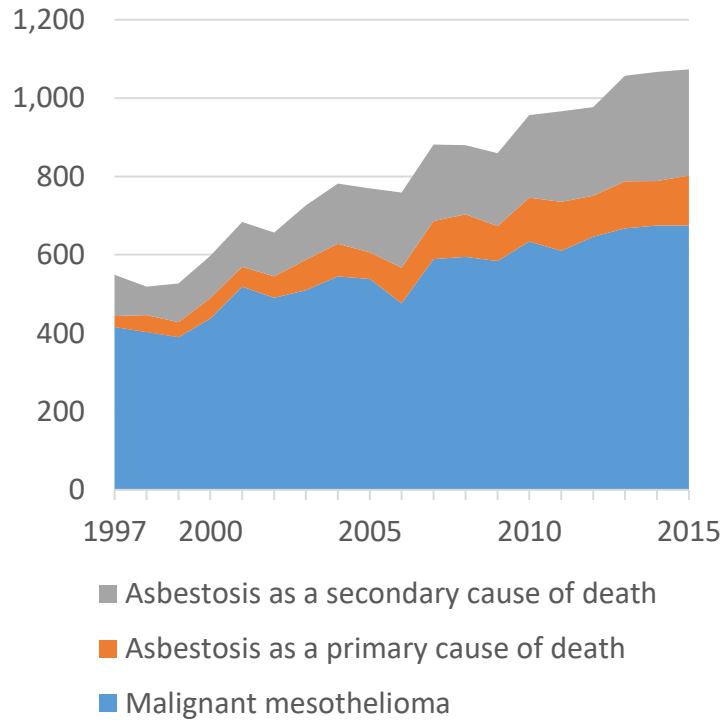
Malignant mesothelioma – primary cause of death



Asbestosis and malignant mesothelioma – primary cause of death



Asbestosis and malignant mesothelioma – primary cause of death + asbestosis - secondary cause of death



Mapping incidence of malignant mesothelioma in NSW (Linton et al. 2017)

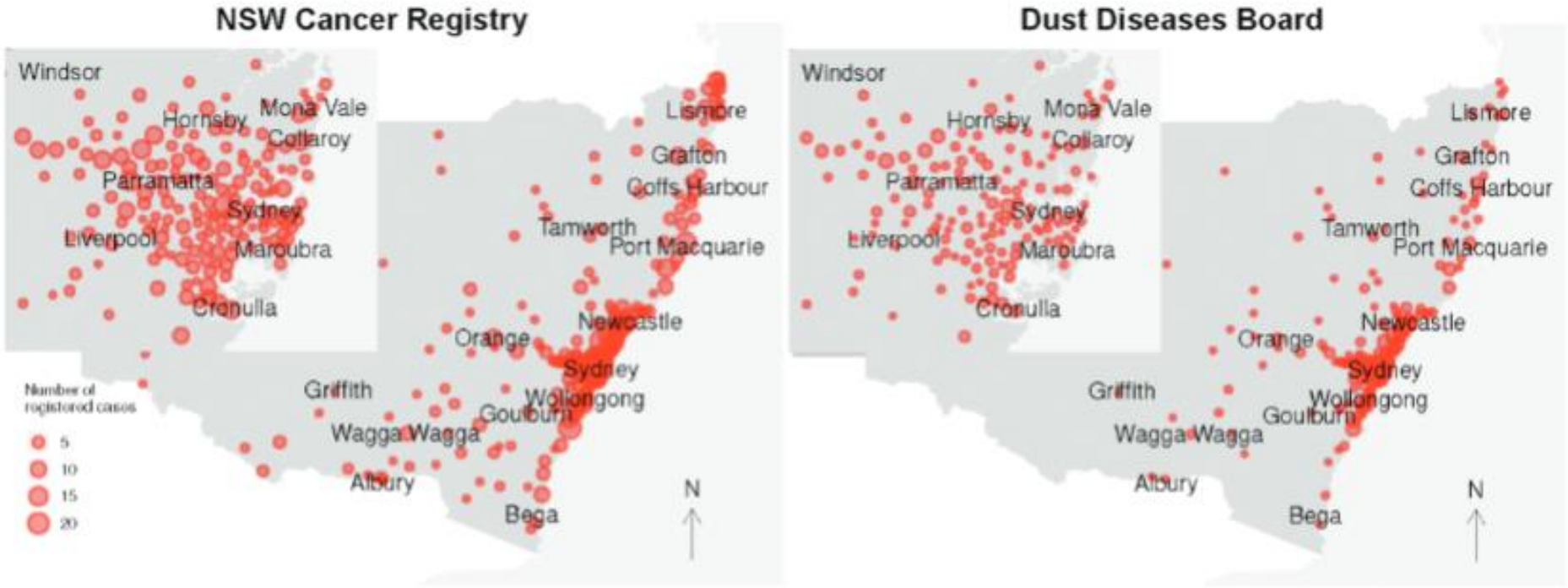
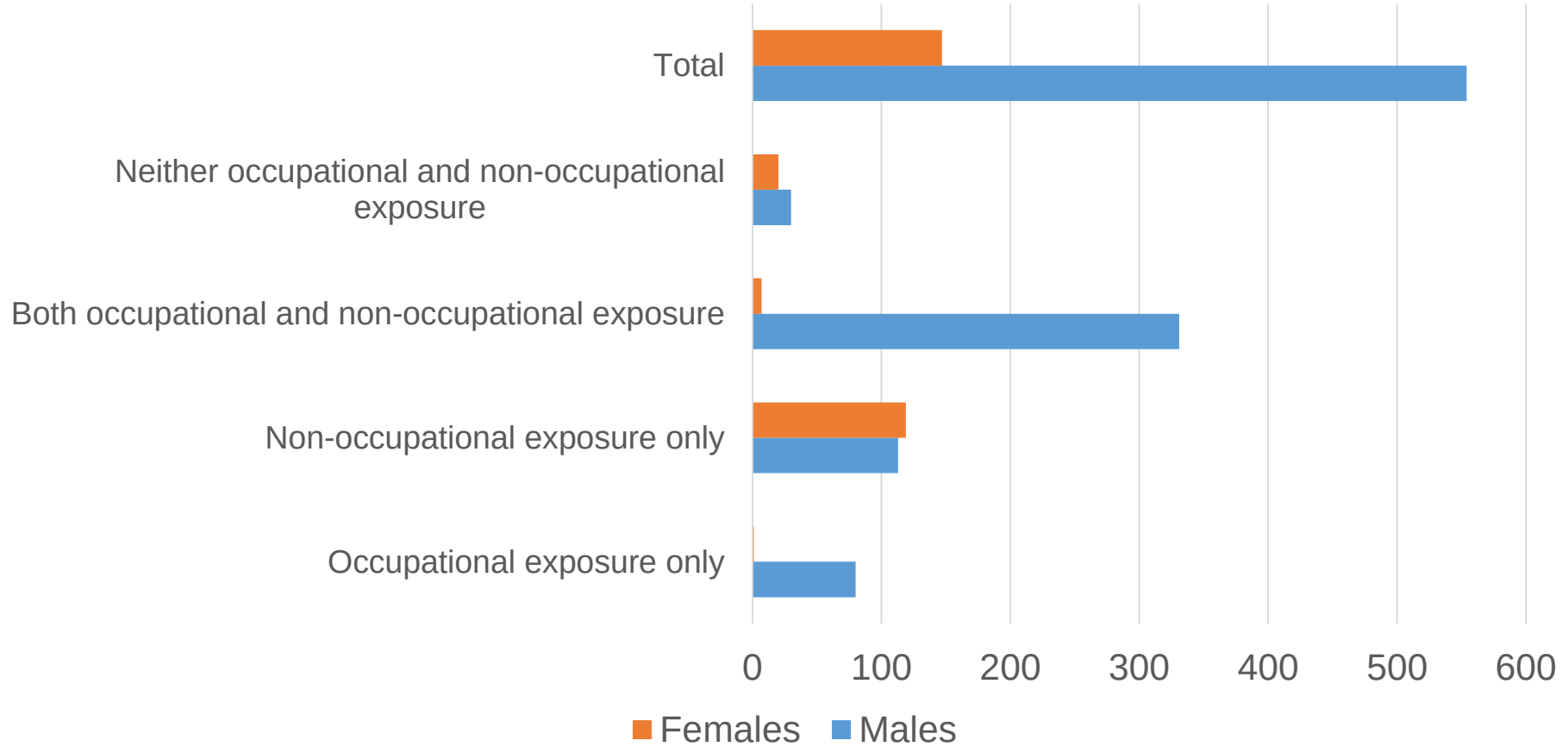


Figure 1 Distribution of malignant pleural mesothelioma (MPM) diagnoses across New South Wales (NSW) between 2002 and 2009 according to NSW Cancer Registry and NSW Dust Diseases Board.

Occupational and non-occupational asbestos exposure for mesothelioma in Australia – Australian Mesothelioma Registry data (September 2017)



What is happening in Australia and internationally on public health research? (R-T Lin et al., 2018)

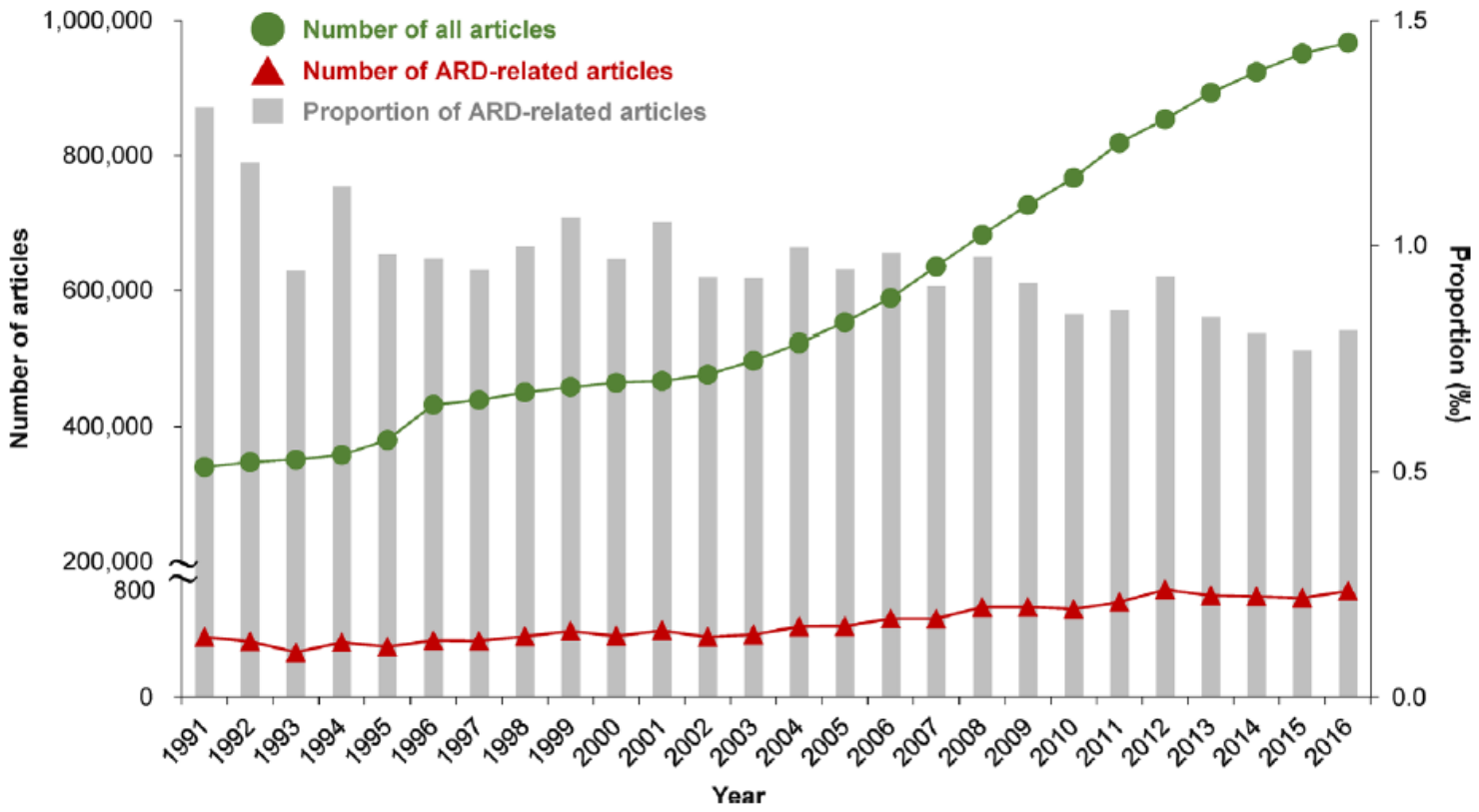


Figure 1 Trend in the number and proportion of scientific articles from 1991 to 2016. ARD-related articles=articles with a theme of asbestos and ARDs. Articles were defined as articles or reviews belonging to any of the three research areas (see online supplementary file, table S1) in InCites (Clarivate Analytics).[17] ARD, asbestos-related diseases.

What is happening in Australia and internationally on public health research? (R-T Lin et al., 2018)

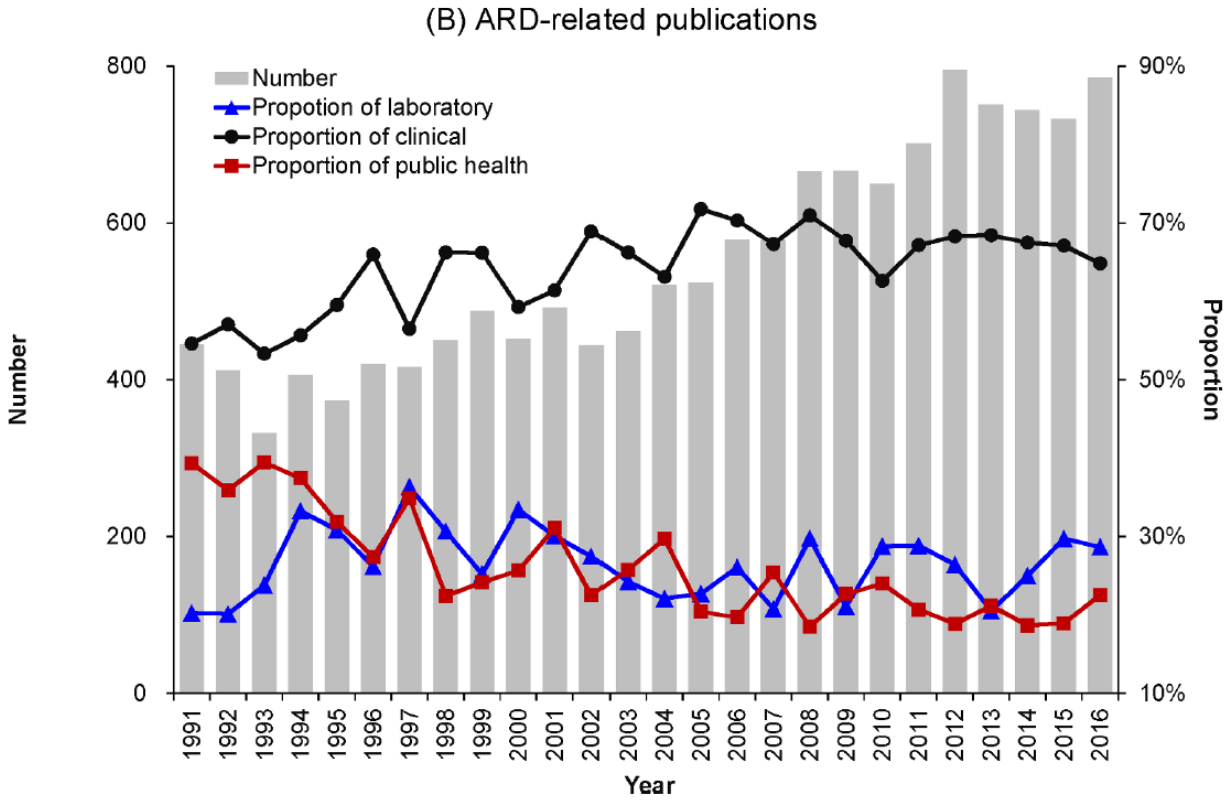


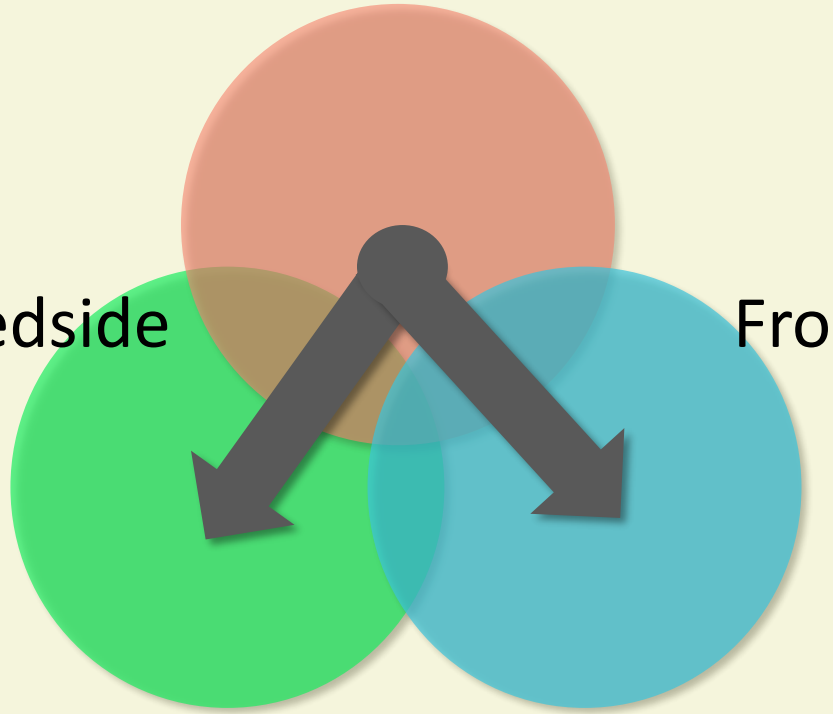
Figure 2 Trend in the number and proportion of articles by research area. ARD-related publications=articles with a theme of asbestos and ARDs. Articles were defined as articles or reviews belonging to any of three research areas (see online supplementary file, table S1) in InCites (Clarivate Analytics).[17] ARD, asbestos-related diseases.

Potential future research directions

- Limiting the further decline of asbestos-related public health research in Australia and internationally
- Linking laboratory data with public health data to better understand causal associations between asbestos fibre types and the total burden of asbestos-related disease
- Linking hospitalisation data with public health data to better understand treatment and outcomes for people diagnosed with asbestos-related disease
- “Deeper dive” of primary and second death and hospitalisation data for asbestosis
- Taking a public health approach to understanding silicosis exposure and links to cancer

Laboratory

From Bench to Bedside



From Bench to Public

Clinical

Public Health / Prevention

From Bench to Public: Another Direction of Translational Research

Ken Takahashi, Yuen Cheng, Matthew Soeberg
Asbestos Diseases Research Institute

ADRI
Asbestos Diseases Research Institute

@AsbestosSafety Asbestos Safety and Eradication Agency asbestossafety.gov.au/asbestosconference2018

ASBESTOS: the next national plan

Proactivity, prevention, planning

Workshop 5

Research Directions

#2018ASEACONF

Australian Government
Asbestos Safety and Eradication Agency

NSW

Thuroona Services

Research Directions

NHMRC Environmental and Public Health Guidelines: Translating research into advice and guidelines

Dr Elaine Stone, Assistant Director
Public Health Section
National Health and Medical Research Council

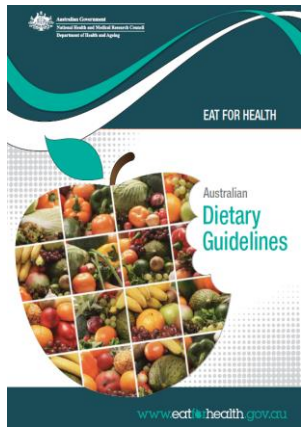
Australian Government
National Health and Medical Research Council

NHMRC

BUILDING A HEALTHY AUSTRALIA

Core NHMRC public and environmental health areas

- Drinking Water quality (includes information on asbestos in water)
- Recreational Water quality
- Lead advice (blood lead levels)
- Water fluoridation
- Nutrition
- Risks of alcohol consumption
- Infection prevention and control



Who asks us to do the work?

Chief Medical Officer; State and territory Chief Health Officers'; Department of Health

- Lead Statement,
- Water Quality guidelines
- Dietary and Alcohol Guidelines

Health Minister directive

- Air quality in traffic tunnels

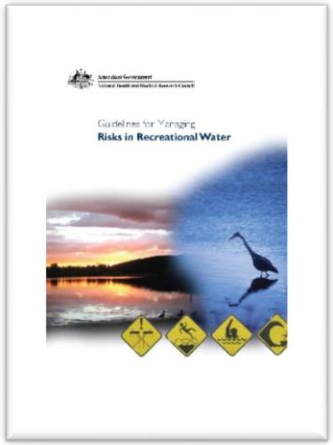
Council of NHMRC

- Fluoridation Public Statement
- Windfarms Statement

CEO Statement

- E-Cigarettes

Mainly funded through cost recovery: contributions from state and territory health departments and Australian Government Departments.



Recent environment health resources

Water Quality

Drinking water

- Australian Drinking Water Guidelines (rolling review since 2011)

Recreational Water

- Guidelines for Managing Risks in Recreational Water (2008)

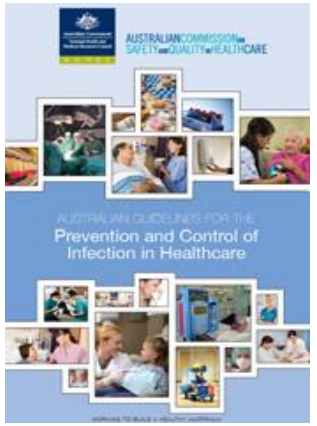
Lead Exposure

Public Statement and Information Paper: Evidence on the effects of lead on human health (2016)

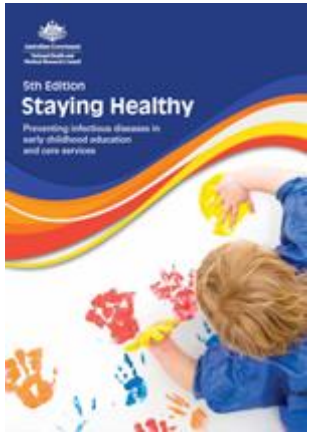
Wind Farms

Public Statement and Information Paper (2015)

Recent public health resources



- **Australian Dietary Guidelines** (2013)
- **Alcohol guidelines** (2009)
- **Fluoridation** (2017)
  - Public Statement: Water fluoridation and human health in Australia (2017)
- **Infection prevention**
  - Infection Control Guidelines (2010)
  - Staying Healthy in Childcare guidance (2012)



Development/Updating Environmental Health Advice and Guidelines:

A
- Establish an expert advisory committee

B
- Prioritise areas for review and develop the scientific questions
- Consideration of other national and international guidelines
- Conduct reviews of the evidence & gather other 'additional' evidence

C
- Quality processes: methodological review, expert review

D
- Develop/update advice / guideline

E
- Undertake public consultation

F
- Finalise Advice/Guideline and receive sign off by Council and CEO

NHMRC

Environmental Health Example: Australian Drinking Water Guidelines

- ADWG provides national guidance for drinking water quality
  - Includes recommendations that support consistency and harmonisation across state and territories
  - Are guidelines, NOT mandatory standards
- Standards are then developed by state and territory health regulators based on the information provided by the ADWG
- Health Based (Related) Guideline Values for each chemical represent NHMRC recommendation for ensuring safe drinking water
- Includes a chemical fact sheet on asbestos

Australian Drinking Water Guidelines- Chemical fact sheet for asbestos (1996)

- ADWG Asbestos fact sheet (1996) – At the time was insufficient data to develop a health based guideline value for asbestos in drinking water.
- Concluded that unlikely that the numbers of asbestos fibres present in most drinking water supplies would be a health concern.
- US EPA has guideline value of 7 million fibres per litre
- WHO (2003) concluded that there is no need to establish a guideline for asbestos in drinking-water. Based on
    - A lack of association between ingestion of asbestos in drinking-water and increased cancer risk in epidemiological studies
    - Feeding studies in animals have not consistently increased the incidence of tumours of the gastrointestinal tract.

NHMRC funded health related research into asbestos

- Since 2000 NHMRC has funded over $30 million on asbestos related research

- Most NHMRC research schemes are Investigator initiated research (with exception of targeted calls for research) and under go a competitive peer review process which means that the amount of research funded depend on the quality of the applications.

Funded research Include:

- Bernie Banton Fellowships (on mesothelioma and asbestosis)
- National Centre for Asbestos Research (Centre of Research Excellence)
- Project Grants (Investigator initiated)

NHMRC funded health related research into asbestos

Grant Type	2009	2010	2011	2012	2013	2014	2015	2016	2017
Career Development Fellowships	$94,250	$96,040	$97,769		$71,841	$79,593	$87,781	$199,810	$226,931
Centres of Research Excellence	$400,000	$400,000	$1,547,966	$931,663	$869,595	$963,529	$1,255,939	$505,005	$512,504
Postgraduate Scholarships	$22,677	$54,875	$111,165	$97,778	$66,894	$53,837	$50,257	$6,012	
Practitioner Fellowships	$127,450	$129,871	$132,208	$45,654				$113,844	$115,438
Project Grants	$762,260	$1,185,250	$1,139,752	$839,087	$688,624	$1,000,680	$1,723,474	$1,281,908	$856,119
Targeted Calls for Research	$1,539,047	$948,660							
Total	**$2,945,684**	**$2,814,695**	**$3,028,860**	**$1,914,182**	**$1,696,954**	**$2,097,639**	**$3,117,450**	**$2,106,580**	**$1,710,991**

- For more information on NHMRC funding opportunities https://nhmrc.gov.au/funding

Challenges in assessing environmental health evidence

- Historically depended significantly on expert opinion.
- Moving towards an evidence-based approach.
- Need to consider epidemiological and toxicological data (often animal studies), rarely Randomised Controlled Trials (RCTs).
- Consideration of peer reviewed and grey literature (e.g technical reports, Water RA project reports, other guidelines)
- Systematic review processes, evaluating data quality and rating the level of evidence are relatively new concepts.
- When do we not need systematic review and when is narrative review/literature review adequate?

Australian Drinking Water Guidelines – Recent Examples of developing environmental health guidance

- Lanthanum Health Based Guideline Value (HBGV) and fact sheet
- Per- and Poly- fluoroalkyl substances (PFAS) fact sheet and HBGV
- Chemical fact sheet review

Example 1 - Development of a Guideline Value and fact sheet for Lanthanum

- Lanthanum is a metallic chemical element used as lanthanum-modified clay in water treatment to reduce algal blooms (e.g. cyanobacteria) on reservoirs.

- NHMRC collaborated with National Industrial Chemicals Notification and Assessment Scheme (NICNAS) to review published literature on lanthanum from (2012- 2015) and considered the NICNAS Secondary Notification Assessment Report (2014) for studies prior to 2012.

- Databases searched: OVID Medline, OVID Embase, AGRIS, AGRICOLA, National Toxicology Program.

Example 1 - Development of a Guideline Value and fact sheet for Lanthanum

- Used Covidence (https://www.covidence.org/) an online tool for conducting systematic reviews.
  - 151 papers were identified and two reviewers screened the references by title and abstract to determine if they met inclusion criteria.
  - 25 were thought to meet the inclusion criteria and full text was examined, of these 2 were relevant. Risk of bias assessed using tool developed by NTP Office of Health Assessment and Translation https://ntp.niehs.nih.gov/pubhealth/hat/review/index-2.html

Example 1 - Development of a Guideline Value and fact sheet for Lanthanum

Inclusion Criteria

- Studies with lanthanum and a control.
- Studies measuring some kind of toxic or health related endpoint (including pharmacological studies on lanthanum in end-stage renal failure cases).
- Studies in humans or non-human mammals (that is, not aquatic invertebrates or fish, etc).
- Studies in whole animals or humans (not *in vitro*, cell cultures).
- Published between November 2012 and October 2015.

Exclusion Criteria

- Non-English language studies.
- Studies that do not contain original data, such as reviews, editorials or commentaries.
- Studies that have not been peer reviewed (e.g. conference abstracts, technical reports, theses/dissertations, working papers from research groups or committees, and white papers).

Calculation of a Guideline Value

- Chemical guidelines are calculated from the **Acceptable Daily Intake** (ADI) = "the amount of a chemical that can be ingested daily by a human over a lifetime without appreciable health risk"
  - Tolerable Daily Intake (TDI) in WHO and European documents
  - Reference Dose (RfD) in US documents
- When human data is not available, the ADI is extrapolated from the next best thing: the **N**o **O**bserved **A**dverse **E**ffect **L**evel (NOAEL) from animal toxicity data (rodents, dogs, primates)
- The NOAEL is the highest dose that produces NO adverse effects
- Uncertainty factors (UF); also called "Safety Factors"(SF) are applied to animal NOAEL to extrapolate ADI for humans.

Calculation of a Guideline Value

- Guideline value calculated as:

$$GV\ (mg/L) = \frac{ADI\ (mg/kg/d) \times body\ weight\ (kg) \times P}{daily\ water\ intake\ (L/d)}$$

- Where
  - Body weight = average body weight in Australia (70 kg) [60 kg in WHO]
  - P = proportion of intake from water (also sometimes referred to as RSC Relative Source Contribution); accounts for exposure from other sources, and usually set at P = 0.1
  - Daily water intake = 2 L/d (based on empirical data) [more in tropics]

Calculating guideline values for Lanthanum

- Short-term, long-term and sub-lethal (*e.g.*, carcinogenicity, reproductive toxicity, neurotoxicity) studies in mice, rats, rabbits, dogs, goats
- Neurobehavioural changes in rat pups was most sensitive endpoint: NOAEL = 0.06 mg/kg bw/d as La^{3+}

$$ADI = \frac{NOAEL}{UF} = \frac{0.06\ mg/kg\ bw/d}{10\ (inter) \times 10\ (intra)} = 0.000\ 6\ mg/kg/d$$

$$\mathbf{GV} = \frac{0.000\ 6\ mg/kg/d \times 70\ kg \times 0.1}{2\ L/d} = \mathbf{0.002\ mg/L}$$

Example 2 - Development of a Guideline Values and fact sheet for Per- and poly-fluoroalkyl substances (PFAS)

- NHMRC used a review from Food Standards Australia New Zealand (FSANZ) and information on methods from National Measurements Institute (NMI) to inform the Fact Sheet.
- FSANZ conducted a review of available literature to determine Tolerable Daily Intakes (TDI) for Per- and poly-fluoroalkyl substances including PFOS, PFOA and PFHxS.
- FSANZ concluded that available human epidemiology data are not suitable to support the derivation of TDI for PFOS or PFOA.
- TDIs based on extensive toxicological databases in laboratory animals

NHMRC

Example 2 - Development of a Guideline Value and fact sheet for Per- and poly-fluoroalkyl substances (PFAS)

- NHMRC worked with its Water Quality Advisory Committee to develop guidelines values for PFOS and PFOA using a TDI established by FSANZ which is based on decreased parental and offspring body weight gains in a multigenerational reproductive toxicity study in rats.

- Calculation based on

  - Body weight = average body weight in Australia (70 kg) [60 kg in WHO]
  - P = proportion of intake from water. Set at P = 0.1
  - Daily water intake = 2 L/day

NHMRC

Calculating guideline values for PFOS

- Example with PFOS based on an TDI of 0.02 µg/kg/d, established by FSANZ (2017):

$$\text{GV (mg/L)} = \frac{\text{TDI (mg/kg/d)} \times \text{body weight (kg)} \times \text{P}}{\text{daily water intake (L/d)}}$$

$$\text{GV (mg/L)} = \frac{0.000\,02 \text{ mg/kg/d} \times 70 \text{ kg} \times 0.1}{2 \text{ L/d}}$$

GV = 0.000 07 mg/L = **0.07 µg/L**

Challenges in assessing environmental health evidence- example Australian Drinking Water Guidelines

- Environmental health usually involves complex issues

e.g. Disinfection by-products in drinking water

- Large number of chemicals >600 DBP exist
- Benefits of disinfection weighed again potential association with adverse effects (microbiological risk).
- Epidemiological evidence limited and poor quality
- Limited detailed toxicological assessments
- Look at surrogates ? Generalise information of groups of DBP?
- Harm vs benefit?

Challenges in assessing environmental health evidence

- What databases capture the relevant literature in environmental health? How to use grey literature?

  e.g. microbial health based targets

- New way of working for experts. Being considered internationally e.g. WHO, National Toxicology Program (USA), Cochrane, evidence based toxicology handbook.
- ADWG - guidelines not legislation so up to states and territories to implement as they see fit.

Example 3- Chemical fact sheet review- updating guideline values

- Part V of the ADWG contain more than 200 fact sheets, most of which have guideline values
- These undergo rolling revisions to ensure they represent the latest scientific evidence
- It is impossible to update all guidelines all the time, so NHMRC Water Quality Advisory Committee (WQAC) has recently developed a screening approach to prioritise revisions.

Challenges in assessing environmental health evidence

- Commencing review of prioritised chemical guideline values including lead, nickel, antimony, cadmium, selenium, copper and some disinfection by-products (e.g THMs).
- As part of review are developing a new methodological framework which can eventually be used for all chemical fact sheets in the ADWG (including asbestos)
- For updating chemical guidelines values are looking to leverage off existing regulatory work
    - Eg TDI, ADI, NOAEL, LOEAL to develop health based guideline values (HBGV)

Thank you

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More information on NHMRC:
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@AsbestosSafety Asbestos Safety and Eradication Agency asbestossafety.gov.au/asbestosconference2018

ASBESTOS: the next national plan

Proactivity, prevention, planning

Workshop 5

Research Directions

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