The use of diagnostic FFPE material in cancer epidemiology research

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Overview

- Who we are
- What we do
- Why FFPE (Formalin Fixed Paraffin Embedded)
- Challenges of using FFPE
- Future uses of FFPE
- Gain Support for collections in the future

PEDIGREE Cancer Study

Pathology Epidemiology, DNA, Informatics and Genetics: a Research Enabling Enterprise

(http://www.pedigree.org.au)

PEDIGREE Cancer Study

Endeavours to generate evidence so that cancer genomics can be used to prevent cancer through:

- Leading and contributing to, the discovery of new genomic and molecular advances that help identify people at genetic risk of cancer
- Using epidemiological and epigenetic data to find the environmental and lifestyle exposures relevant to people at genetic risk of cancer
- Develop novel statistic and high performance computing methods
- Nurturing and building the expert multi-disciplinary research team
- Curating and building all aspects of the resource
- Growing national and international collaborators

The Bio-repository

Bio-Specimen Processing	Sample Storage	Tissue Culture	Resource for Research
Processing Blood Saliva Histology	1 Million+ Frozen Blood Fractions 35 000 Saliva 10 000+ FFPE Cases (slides) 150 000 Guthrie Cards (duplicates in 2 locations)	Cell Lines	Active

MCCS

Colon
Prostate
Breast
Kidney
Gastric

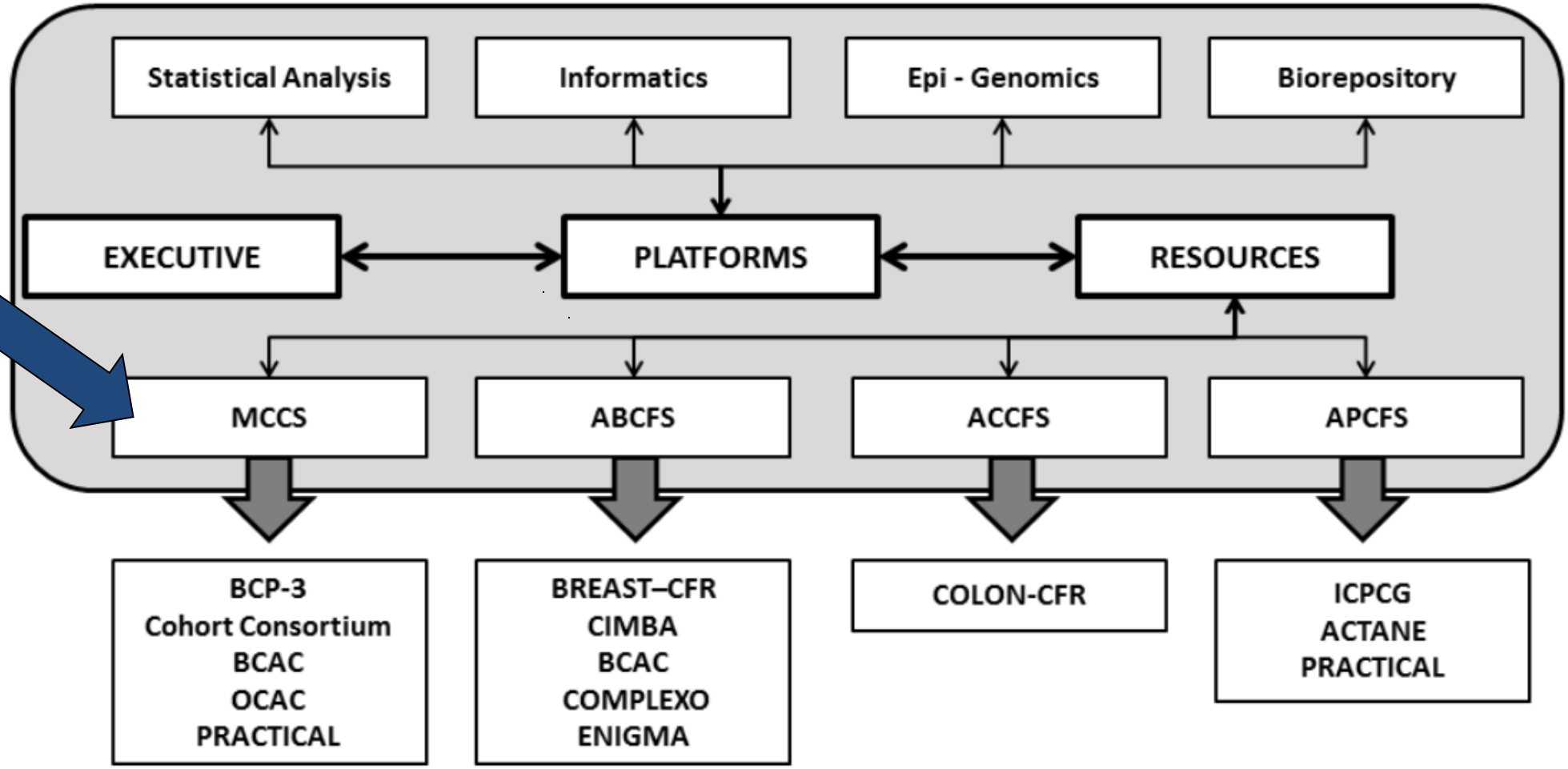
ABC Study

Cancer Council Victoria

PEDIGREE

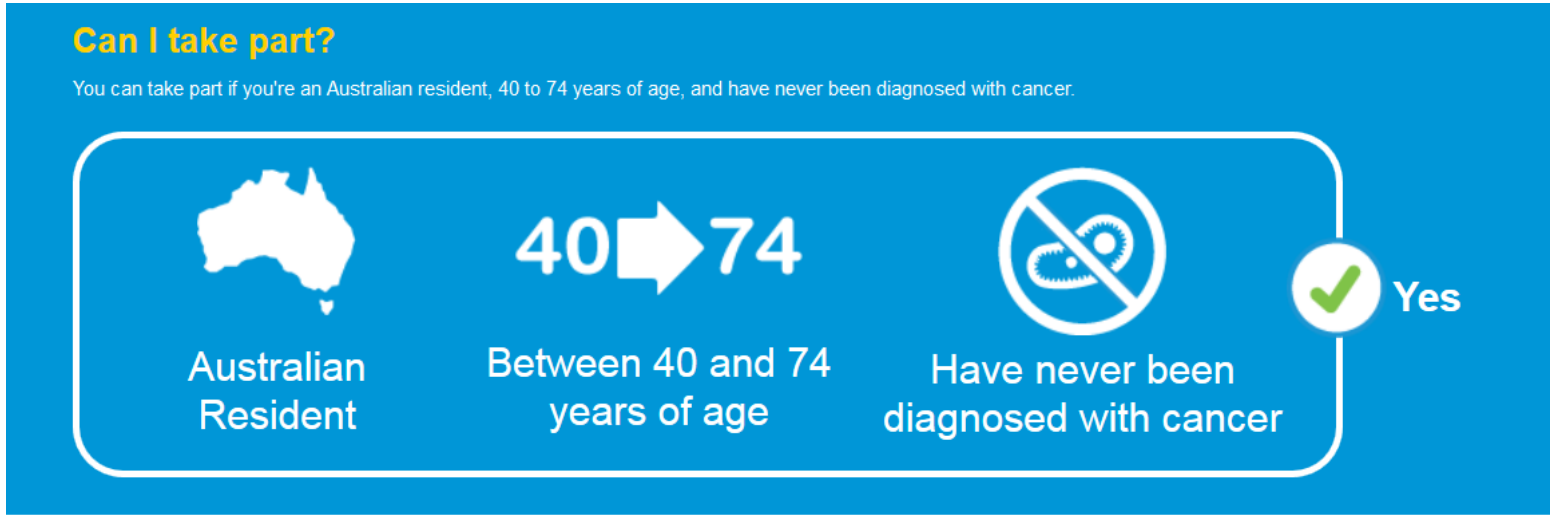
Pathology, Epidemiology, DNA, Informatics and Genetics:
A Research Enabling Enterprise

Structure



International collaborations

ABC Australian Breakthrough Cancer Study



- 11 000 / 20 000 blood samples
- 35 000 / 50 000+ saliva samples
- Future – New Incidents of Cancer

Study Cycle

Study Development

Funding - Australia and International(NIH)

Ethics
- Hrec
- Site Specific Ethics

Participants
- Joining a Study
- Cancer Registries
- Family Cancer Clinics

Recruitment (up to 1 year)
- Informed Consent
- Questionnaire
  - Lifestyle
  - Family History
  - Clinical History

Blood /Saliva Collection (Base Line)

± Tissue Collection

Pedigree

± Pathology Review

Molecular Testing

Data / Publication

Translational Pathology Outcomes

Extension

Follow Up (FUP) 5 years

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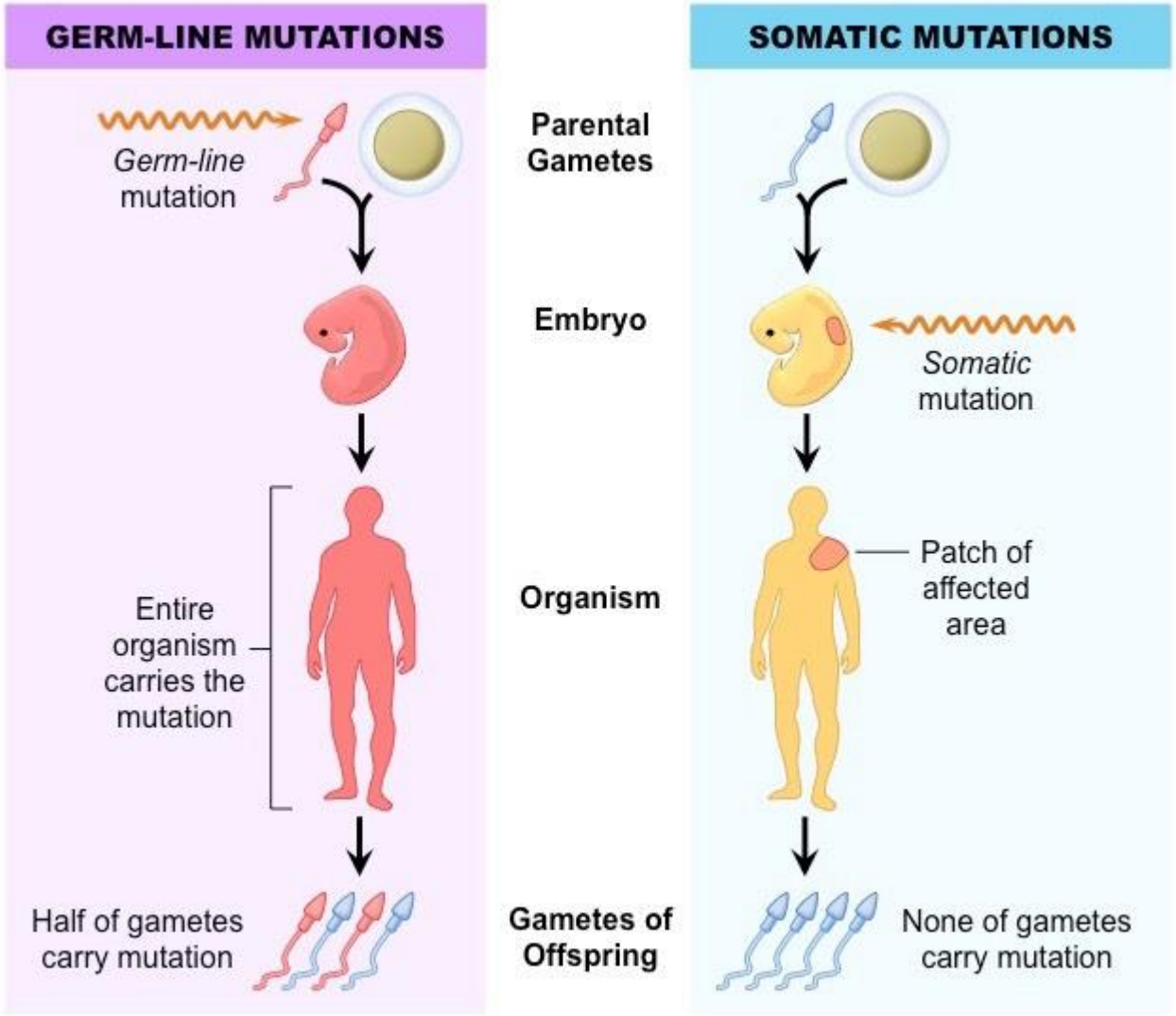
Study Cycle – Follow Up

- Study Modification
- Funding - Australia and International(NIH)
- Ethics
  - Hrec
  - Site Specific Ethics
- Participants - Targeted Family Members
- Re- Recruitment (up to 1 year)
  - New Informed Consent
  - Questionnaire
    - Lifestyle
    - Family History
    - Clinical History
- Blood /Saliva Collection (FUP)
- New Incident Tissue Collection
  - Cancer Registry Searches
- Death Registry Searches
- Pedigree Updates
- Pathology Review
- Molecular Testing
- Data / Publication
- Translational Pathology Outcomes
- Extension
- Follow Up (FUP2)

Blood and FFPE

Blood
Saliva
“Normal” Tissue

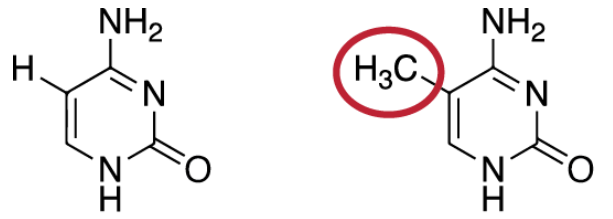


FFPE

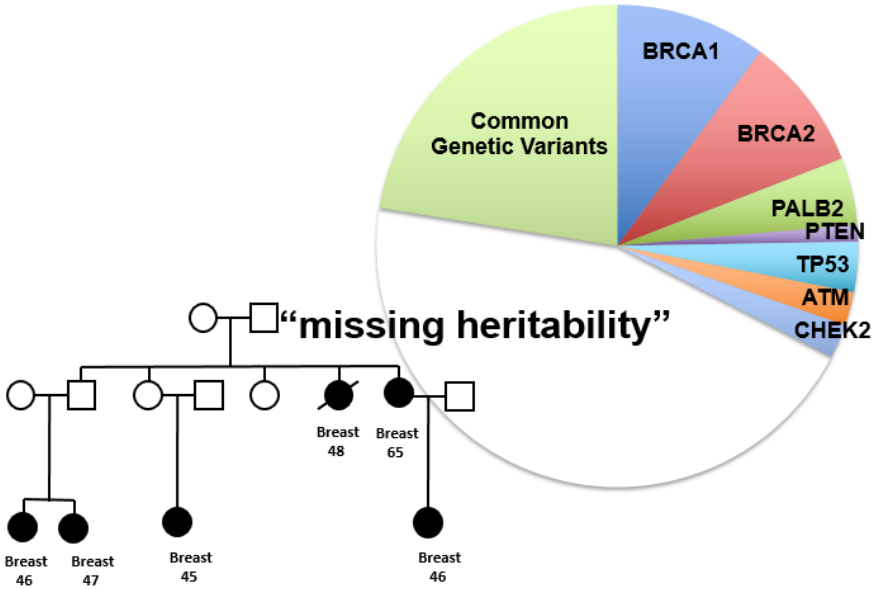
Genetic and Epigenetic Testing

- Epigenetic Factors
  - Methylation
- Genetic Factors
  - New Genetic Variants

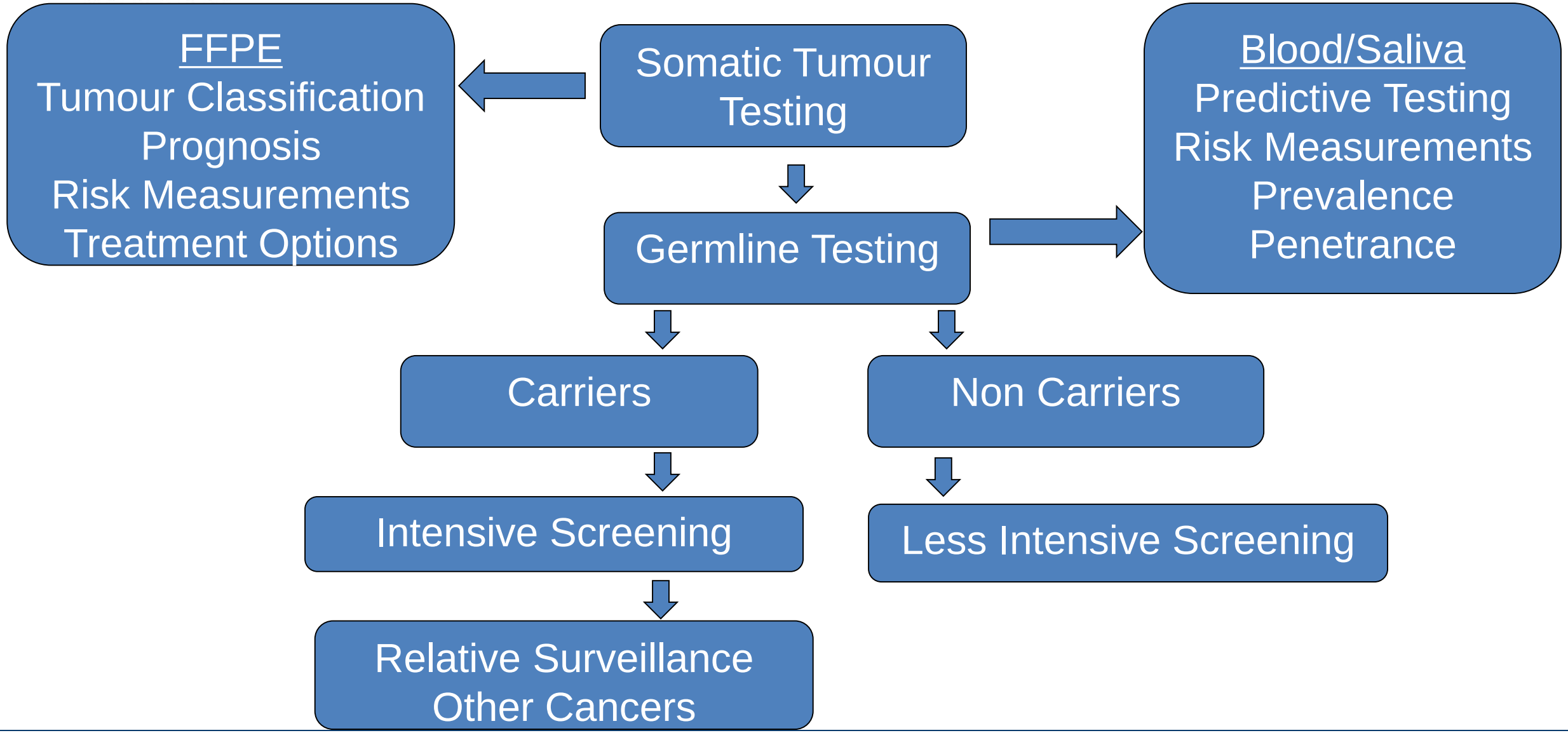
- IHC
  - Screening Tool
  - Prognostic Markers

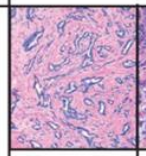
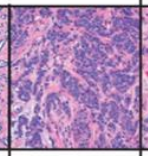
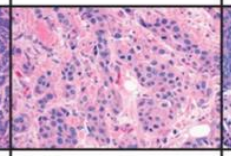
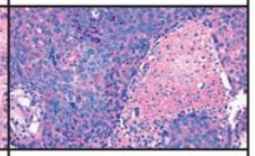
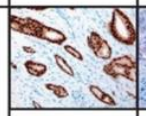
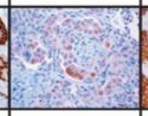
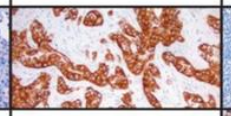
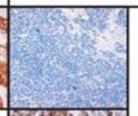
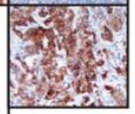


Cytosine methylated Cytosine



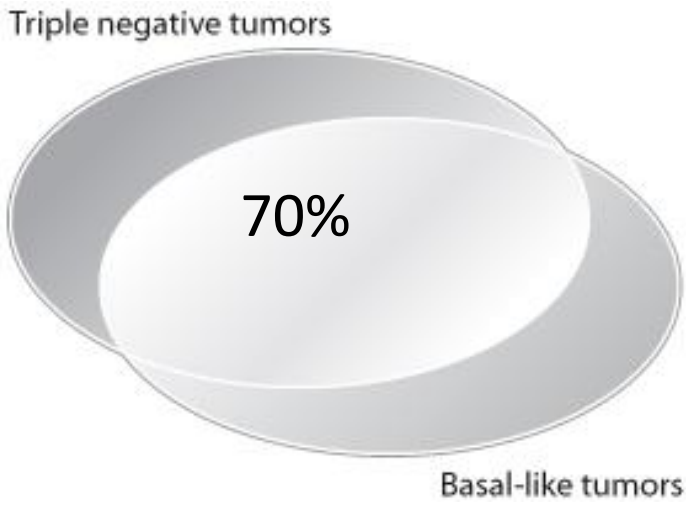
Translational Pathology - Outcomes



Molecular Subtype	Luminal (A and B)		HER2	Basal
Genetic profile	↑ Luminal CKs and ER-related genes (A>B) B↑ in proliferation-related genes		↑ HER2-related genes	↑ Basal CKs
Histologic correlates				
	A Lower-grade ER+	B Higher-grade ER+	High-grade, +/– apocrine features	High-grade, sheet-like, necrosis, inflammation *See exceptions
Surrogate markers				 ER/PR– HER2–  CK5/6+/– EGFR+/–
	A Strong ER+, PR+/–, HER2–, low Ki67	B Weaker ER+, PR+/–, HER2+/–, ↑ Ki67	HER2+, +/– ER/PR	
Prognosis	Good	Intermediate	Worse	Worse
Response to chemotherapy	Lower	Intermediate	Higher	Higher
Targeted therapies	Hormone therapies		HER2-targeted therapies	Currently investigational

Molecular Subtypes of Breast Cancer

Luminal A 40%
Luminal B 20%
Her2 Enriched 15-20%
Basal 15-20%
- Basal Like
- Triple negative (BRCA1 80%)



nature medicine

The consensus molecular subtypes of colorectal cancer

Justin Guinney[1,21], Rodrigo Dienstmann[1,2,21], Xin Wang[3,4,21], Aurélien de Reyniès[5,21], Andreas Schlicker[6,21], Charlotte Soneson[7,21], Laetitia Marisa[5,21], Paul Roepman[8,21], Gift Nyamundanda[9,21], Paolo Angelino[7], Brian M Bot[1], Jeffrey S Morris[10], Iris M Simon[8], Sarah Gerster[7], Evelyn Fessler[3], Felipe De Sousa E Melo[3], Edoardo Missiaglia[7], Hena Ramay[7], David Barras[7], Krisztian Homicsko[11], Dipen Maru[10], Ganiraju C Manyam[10], Bradley Broom[10], Valerie Boige[12], Beatriz Perez-Villamil[13], Ted Laderas[1], Ramon Salazar[14], Joe W Gray[15], Douglas Hanahan[11], Josep Tabernero[2], Rene Bernards[6], Stephen H Friend[1], Pierre Laurent-Puig[16,17,22], Jan Paul Medema[3,22], Anguraj Sadanandam[9,22], Lodewyk Wessels[6,22], Mauro Delorenzi[7,18,19,22], Scott Kopetz[10,22], Louis Vermeulen[3,22] & Sabine Tejpar[20,22]

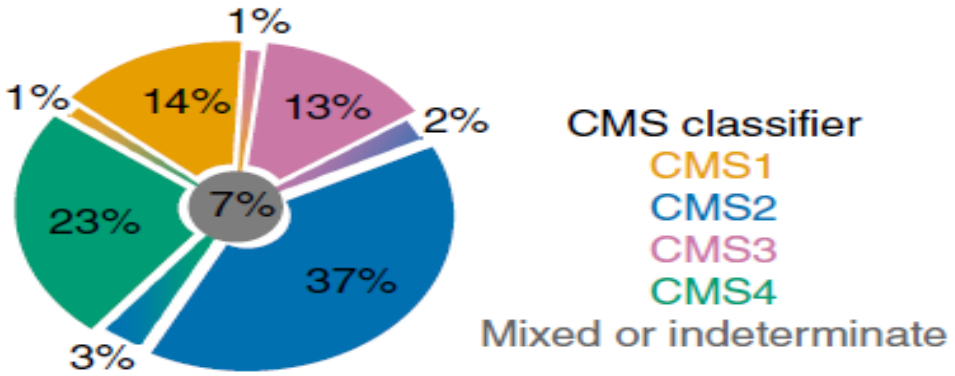
Expression-based classification of CRCs from 4,151 patients

CMS1 – MSI immune
- Hypermutated, MSI-High and strong immune

CMS2 – canonical
- Epithelial, marked WNT and MYC signalling

CMS3 – Metabolic
- Epithelial, metabolic dysregulation

CMS4 – Mesenchymal
- TGF-β activation, stromal invasion, angiogenesis



Translational Pathology – MSI Immune Colorectal Cancer

1)MMR Deficient
2) Immune Rich

↓

Germline Testing
MSI High

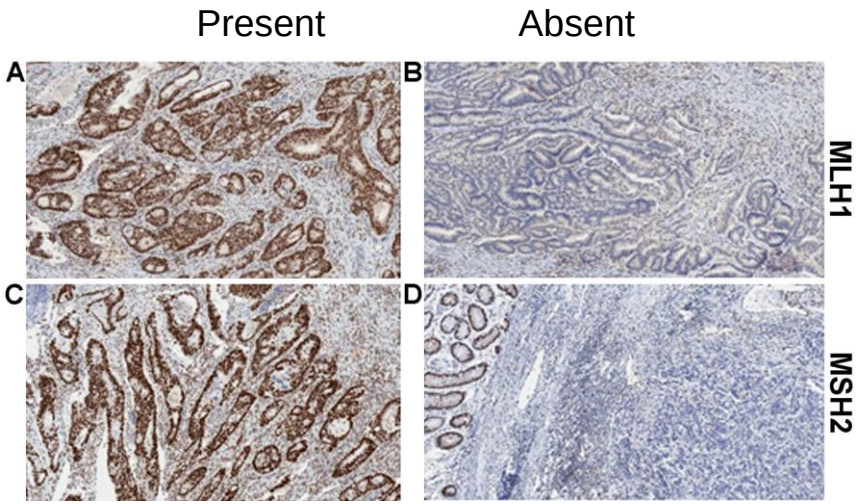
↓

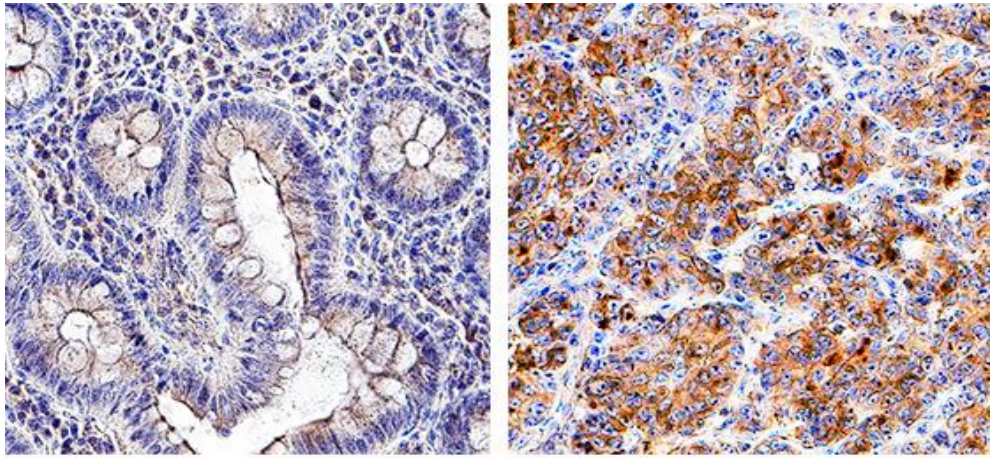
Carrier

↑

Treatment-
Check Point Inhibitor Therapy

- Pembrolizumab (PDL1)





PDL-1 Normal Tissue Cancer

Collection Challenges

- Availability
- Quality
- Storage
- Cost

Collection Challenges

Availability

- Retention times
  - Long cohorts
  - Pedigrees
- Remote archiving
  - Storing and not accessing
- Workload
- Amalgamated / closed laboratories / Custodians?
- Amount of tissue remaining in blocks
- Resistance to access
  - High price to deter
  - Consented / ethics

Table 2 Minimum retention times for anatomical pathology

Please refer to Table 1 (General minimum retention times) for both record and material retention times, unless otherwise specified below.

	Record/material	Minimum retention time
2.1	Slides: Sections of fixed tissue preserved in mounting medium Sections of unfixed tissue not in permanent mounting medium (including immunofluorescence slides)	 10 years†† See General 1.6
2.2	Blocks of tissue embedded in paraffin wax or any other permanent embedding medium	10 years†††‡‡
2.3	Specimens for intra-operative frozen section diagnosis: (i) The original section(s) used for diagnosis, preserved in permanent mounting medium (ii) Residual tissue from which the sections in (i) were prepared, embedded in paraffin (iii) All other blocks of paraffin-embedded tissue from the same Specimen or Specimens from which tissue has been selected for frozen section examination	10 years[8]
2.4	Frozen tissue blocks, including Specimen for immunofluorescence studies	1 month at -70°C or lower
2.5	(i) Containers with no residual tissue (ii) Unblocked tissue from Specimens removed at surgery (iii) Unblocked tissue from Specimens retained at autopsy	1 month 1 month from date of issue of Specimen report 3 months after autopsy unless a limitation is imposed, such as the need to reunite retained Specimens with the body before a funeral has been stipulated by next-of-kin
2.6	Autopsy — registers, report duplicate, blocks and slides, records of tissue and organ disposal	10 years for autopsy other than forensic or other medico-legal autopsy

10 years†††‡‡‡
10 years[8]

Collection Issues

Quality

- DNA
- Antigenicity

– Storage time
– Storage Conditions
  Transport
  Temperature
  Sunlight Exposure UV
– Oxidation
  air
– Hydrolysis
  Endogenous water
  Exogenous water e.g. 4^{o}C Fridge
– Tissue processing time
  Inadequate
  Prolonged
  Fixation time / type

1
Fresh cut sections from Block

2
Sections stored 4 to -20^{O}C

Collection Challenges

Storage

- Bundling
- Dark
- Air tight
- 4ºC

- Lab Storage
  - Limited space
  - Cost

Collection Challenges

Cost

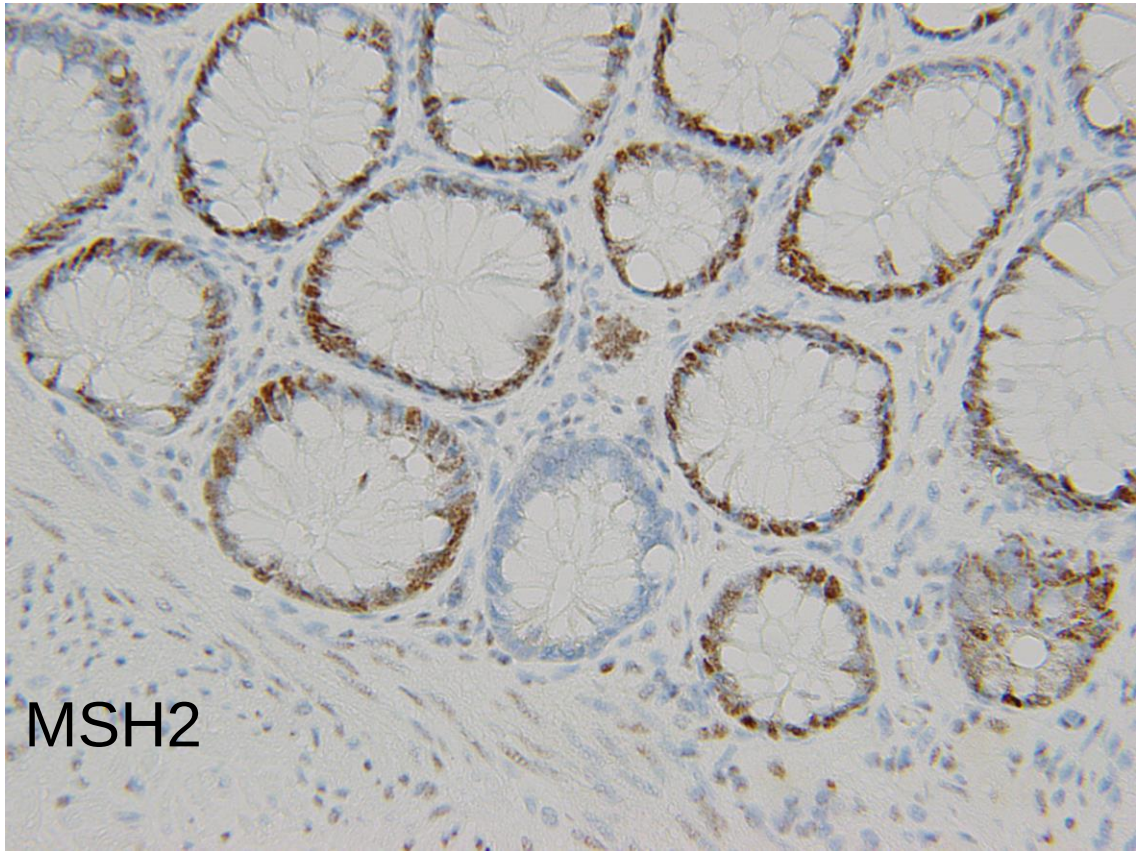
- Cost of collection is rising
- Impact on existing funding
  - Budgets
- Funding decreasing / harder
- Cost recovery

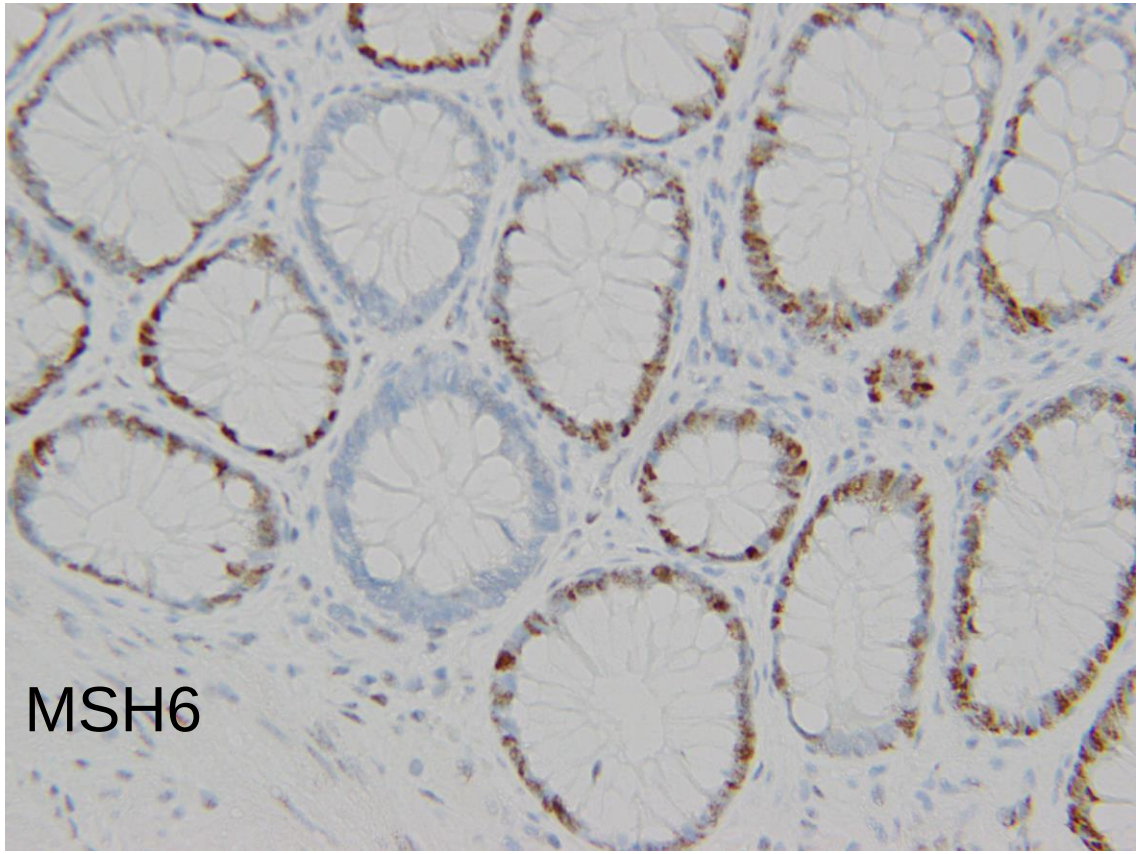
The Future of FFPE Testing

- ?? Independent Repository
- New diagnostic tests
  - Molecular technologies – Cheaper Better
  - Molecular Testing / IHC
  - Research tests = Diagnostic tests = Revenue for laboratories
- IHC to better define areas of interest
  - Tumour heterogeneity
- Collecting more “Normal” and other pre-cancers lesions
  - Predictive tests for patients and families
  - Clinicians driving change

Future – Normal Testing

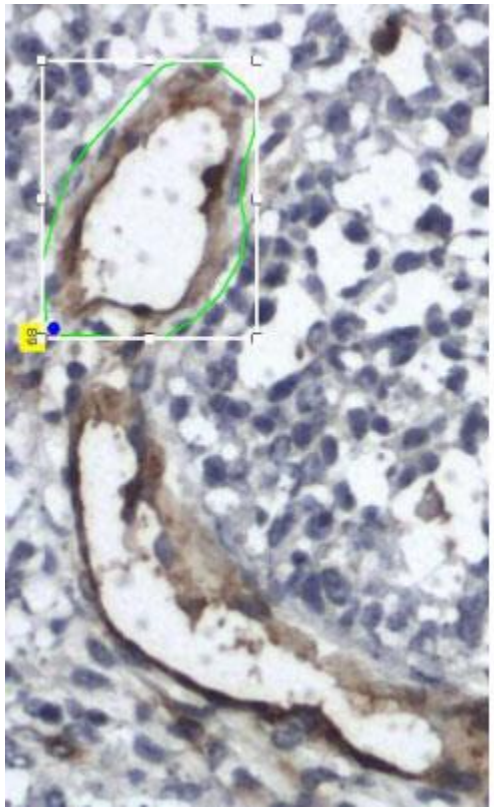
“Normal” Colon from MMR Deficient Carriers

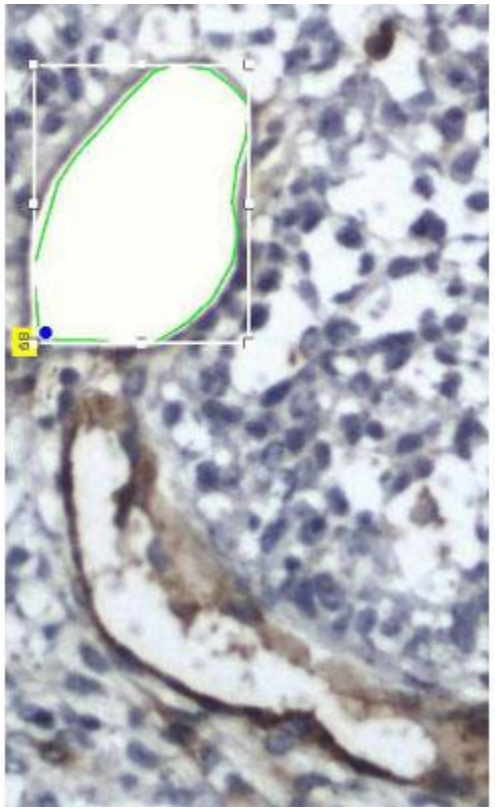




Future - Using IHC for Macro/Micro-Dissection

Laser Capture Micro-Dissection

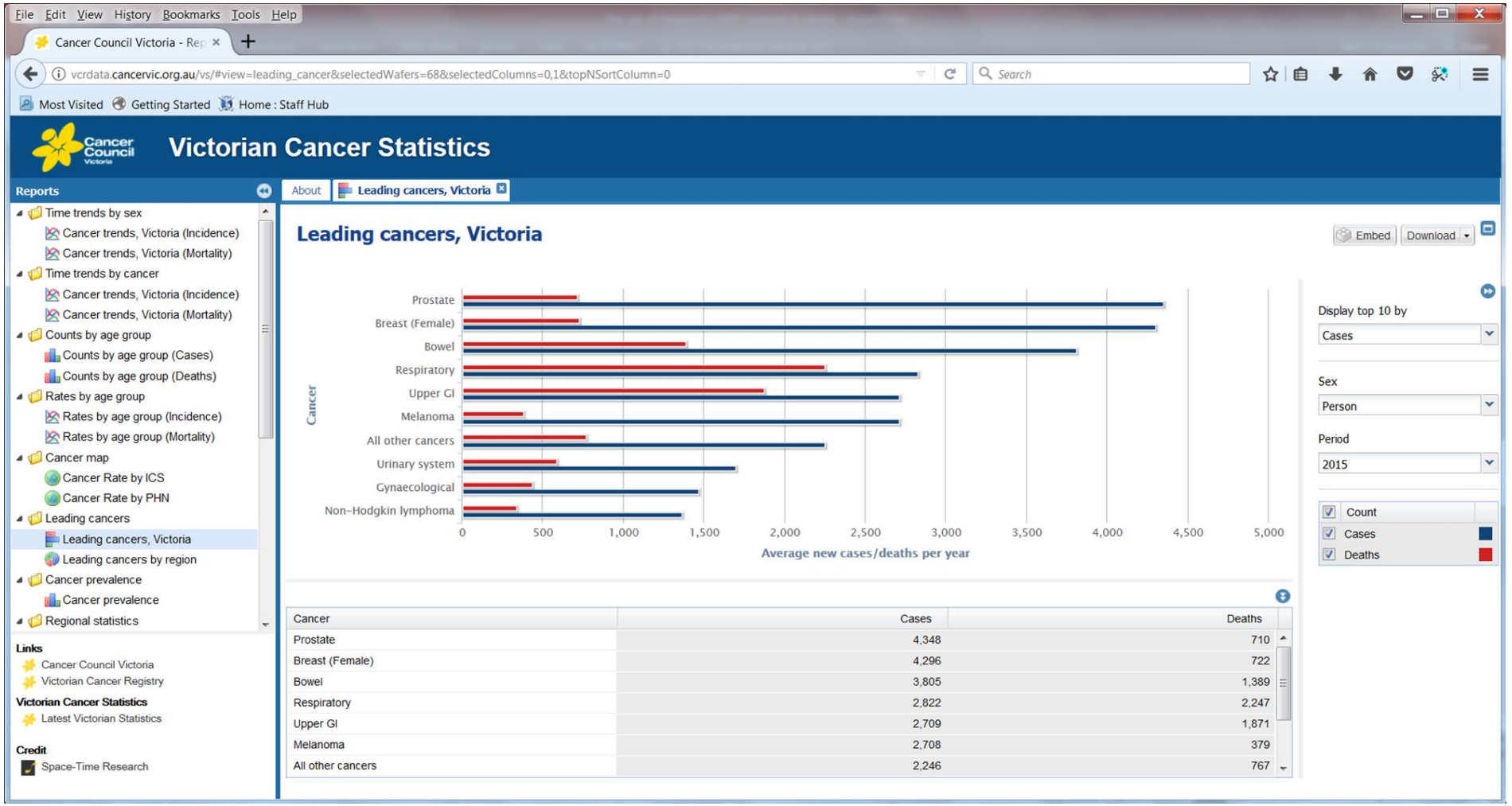




Ducts or Crypts

The Victorian Cancer Registry

- 1982-2015
- Cancer prevalence statistics



http://vcrdata.cancervic.org.au/vs

Concluding

- Focus on FFPE
  - Tumour
    - New gene variants
  - Normal
- Feedback on our requesting
- What can we give back to keep your labs invested
- Our participants want their tissues included in our research.
- Thank you for your contributions to our studies
- Researchers need your support

Acknowledgements

- Our Participants
- Funding bodies
- All laboratories that have provided tissue for our studies