Assessment of the Epigenome – Cohort Scenarios

Univ Prof Martin Widschwendter, MD, FRCOG
University of Innsbruck, Innsbruck, Austria
University College London, London, UK
Karolinska Institute, Stockholm, Sweden

Assessment of the Epigenome – Cohort Scenarios

- Unmet needs in disease prevention (cancer, metabolic, neurologic, etc)
- Rationale for utilising DNA methylation for risk prediction
- DNA methylation and use of surrogate tissue
- Potential cohort biobanks

Unmet needs in disease prevention

Prevention – unmet needs

CARDIOVASCULAR DISEASES

Elevated blood pressure

Risk factor → **Risk prediction** → **Preventive action** → **Risk monitoring**

OTHER DISEASES
(cancer, metabolic, neurologic, etc)

many → **???** → **several, which one is right for you?** → **???**

Risk factors → **Risk prediction** → **Preventive action (primary/secondary)** → **Risk monitoring**

Rationale for utilising DNA methylation for predicting disease risk – example: cancer

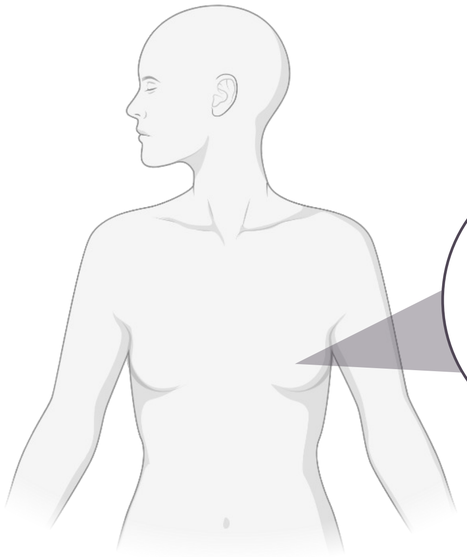
Cancer risk prediction

Genetics

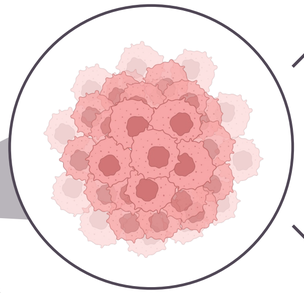
eg. *BRCA1/2* mutations

Environment and lifestyle

Smoking, hormones, chemicals, diet, age, infections, ...



Cancer



Genetic risk prediction
based on mutation analysis or polygenic risk scores
static, captures only proportion

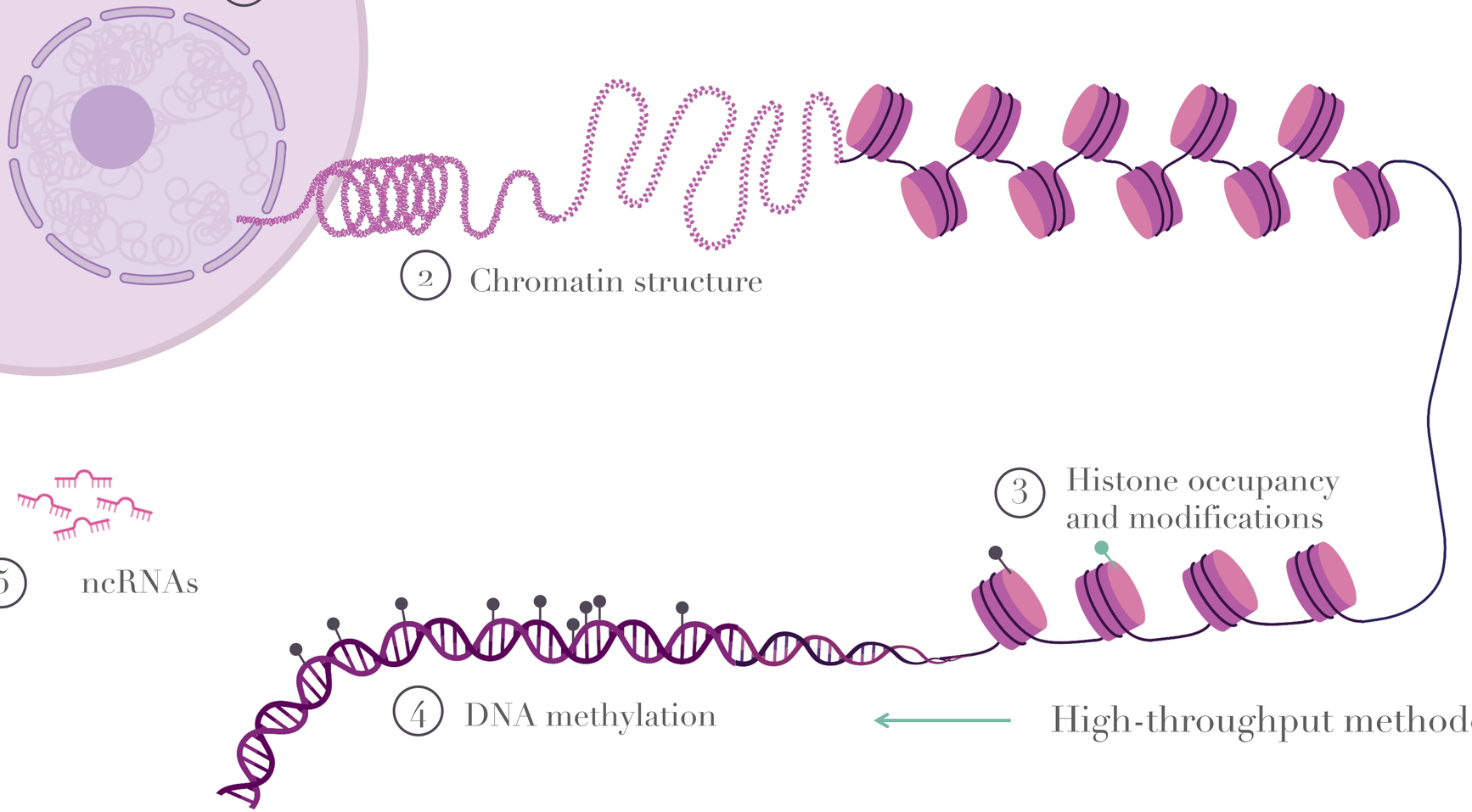
Epidemiological risk prediction
based on environmental factors
does not account for interaction of genetics and environment

To enable cancer risk prediction, we need a tool that can account for both genetic and environmental factors

DNA sequence is determined at birth.
Epigenetics can be influenced by external factors.

DNA methylation

Epigenetics – DNA methylation



1 Domains in the nucleus

2 Chromatin structure

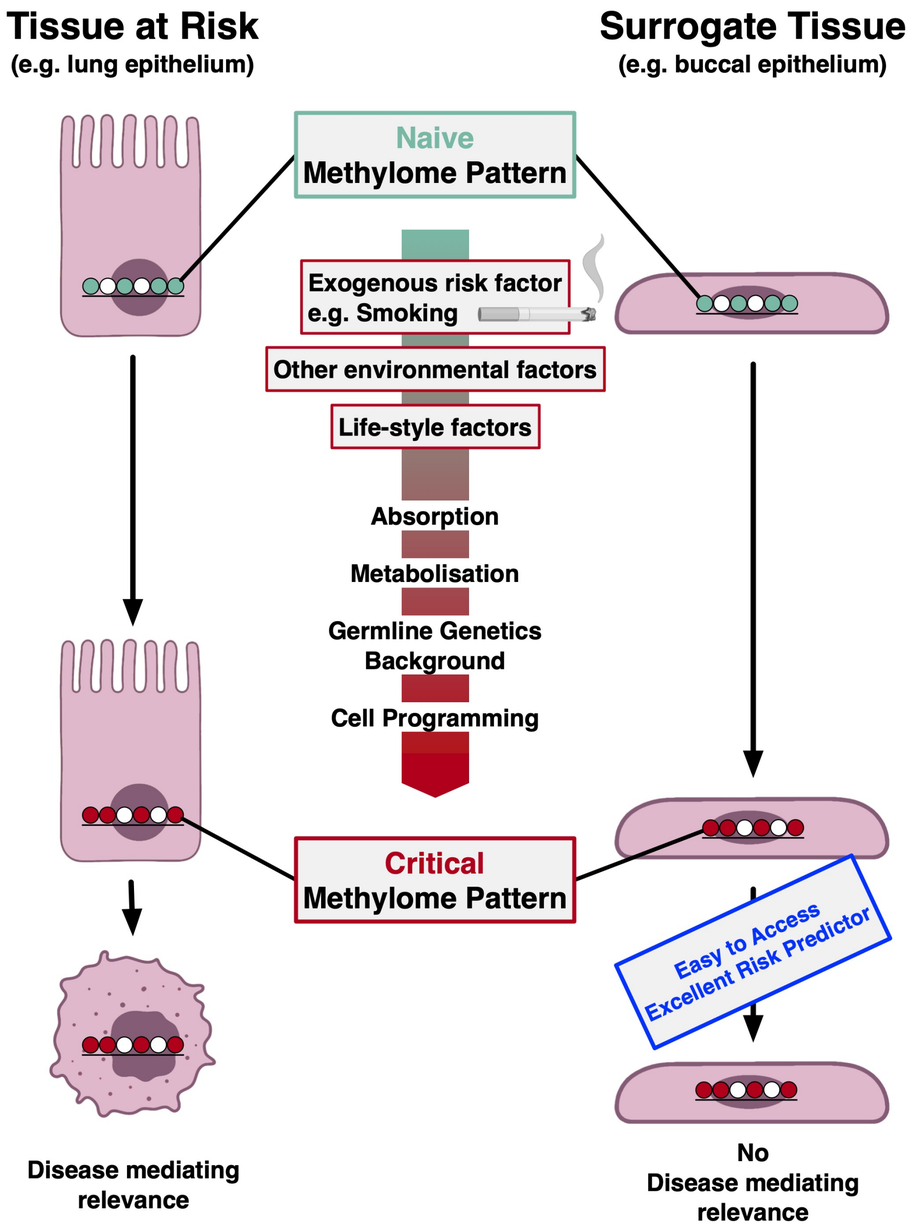
3 Histone occupancy and modifications

4 DNA methylation

5 ncRNAs

High-throughput methodologies available

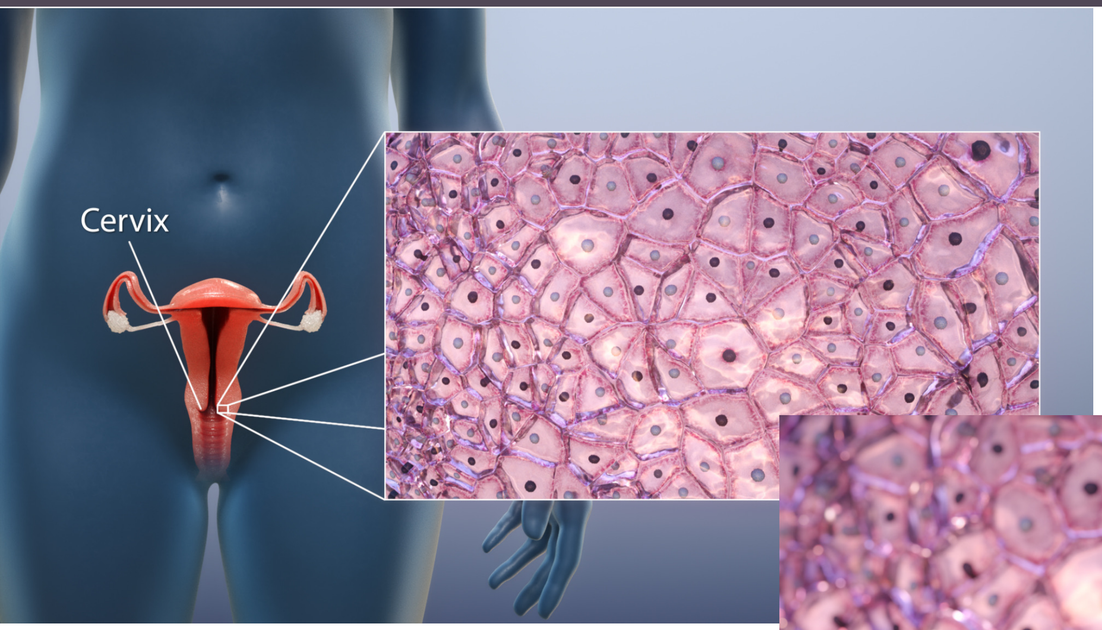
Surrogate tissue

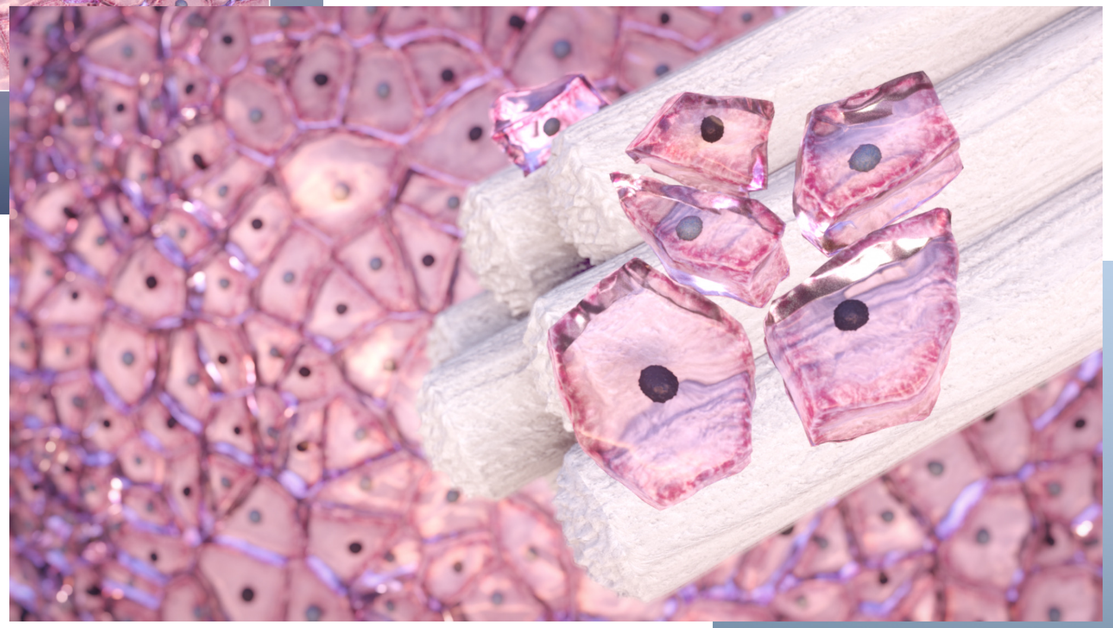


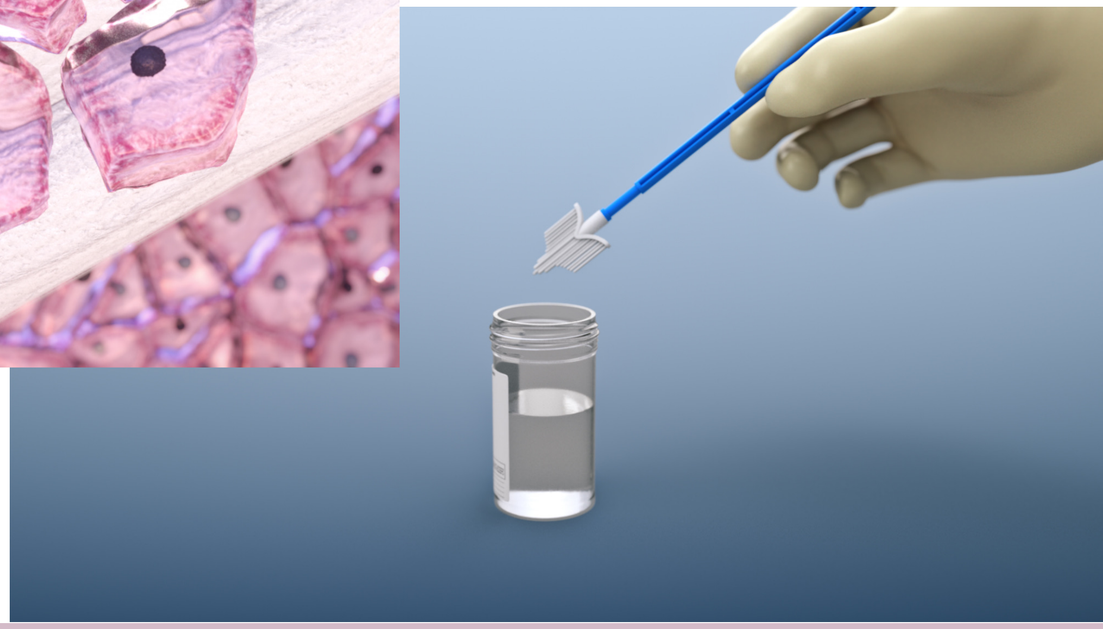
*How does an **ideal surrogate tissue** look like?*

- Easy accessible (ideally self-collected)
- Capturing specific disease pathways
- Variability of features reflective of tissue at risk
- Similar/same embryological origin

Surrogate tissue – easy accessible

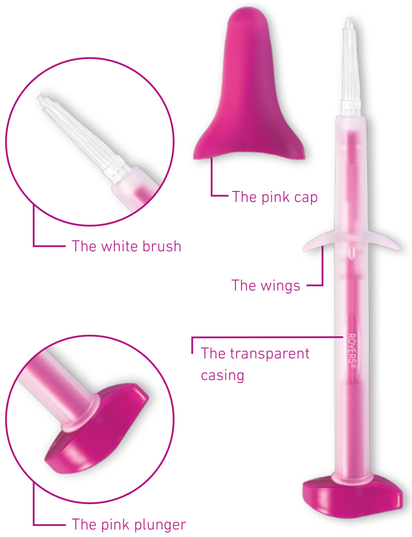






Surrogate tissue – ideally self-sampling is feasible

evalyn® brush



Wash your hands before usage.

Remove the Evalyn Brush from the packaging. Do not throw the packaging away, as it is necessary for sending the Evalyn Brush to the laboratory after usage.

Press the sides of the pink cap with your thumb and index finger to remove the pink cap from the Evalyn Brush. Ensure that you do not touch the white brush of the Evalyn Brush with your hands!

Obtain the sample whilst in a standing position. Assume a comfortable stance (e.g. as if you were about to insert a tampon).

Spread your labia with one hand, and with the other, insert the Evalyn Brush into your vagina until the wings touch your labia.

Hold the transparent casing with one hand, and with your other hand, push the pink plunger in the direction of the transparent casing. You will hear and feel a click when the brush is in the right position with the pink plunger directly against the casing.

Turn the pink plunger five rotations in the same direction. After each rotation, you will hear a click. This helps you count the rotations. After turning the plunger five times, carefully remove the Evalyn Brush.

Hold the transparent casing with one hand, and with your other hand, pull on the pink plunger until the white brush disappears into the casing. When doing so, do not touch the top part of the Evalyn Brush above the wings.

Hold the transparent end to ensure the white brush does not extend again. Place the pink cap back on the Evalyn Brush using your thumb and index finger. You will hear a click when it is properly in place.

Put the Evalyn Brush back inside the packaging.

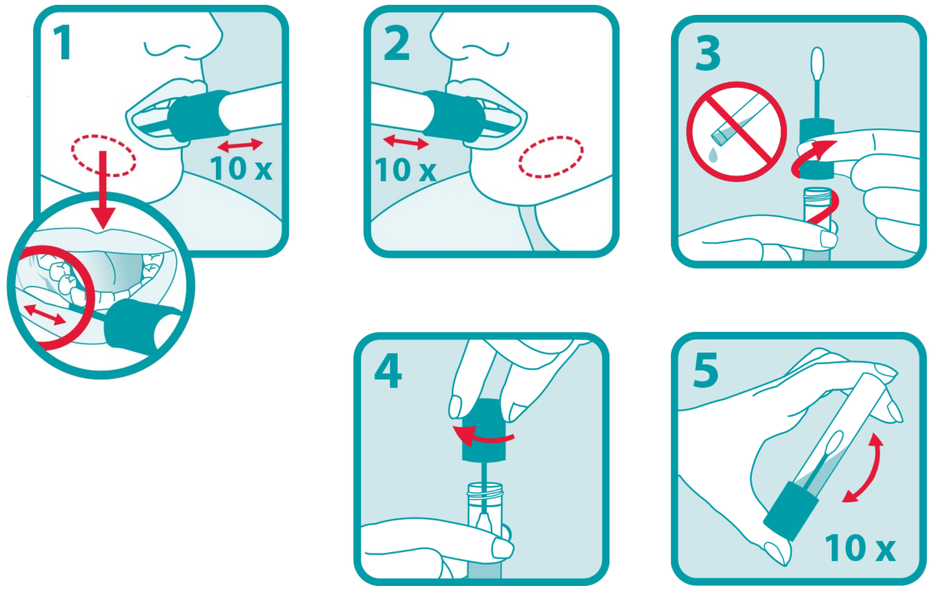
Place the packaging containing the Evalyn Brush into the plastic bag provided and seal it.

Use the return envelope to send the plastic bag containing the Evalyn Brush together with other required information.

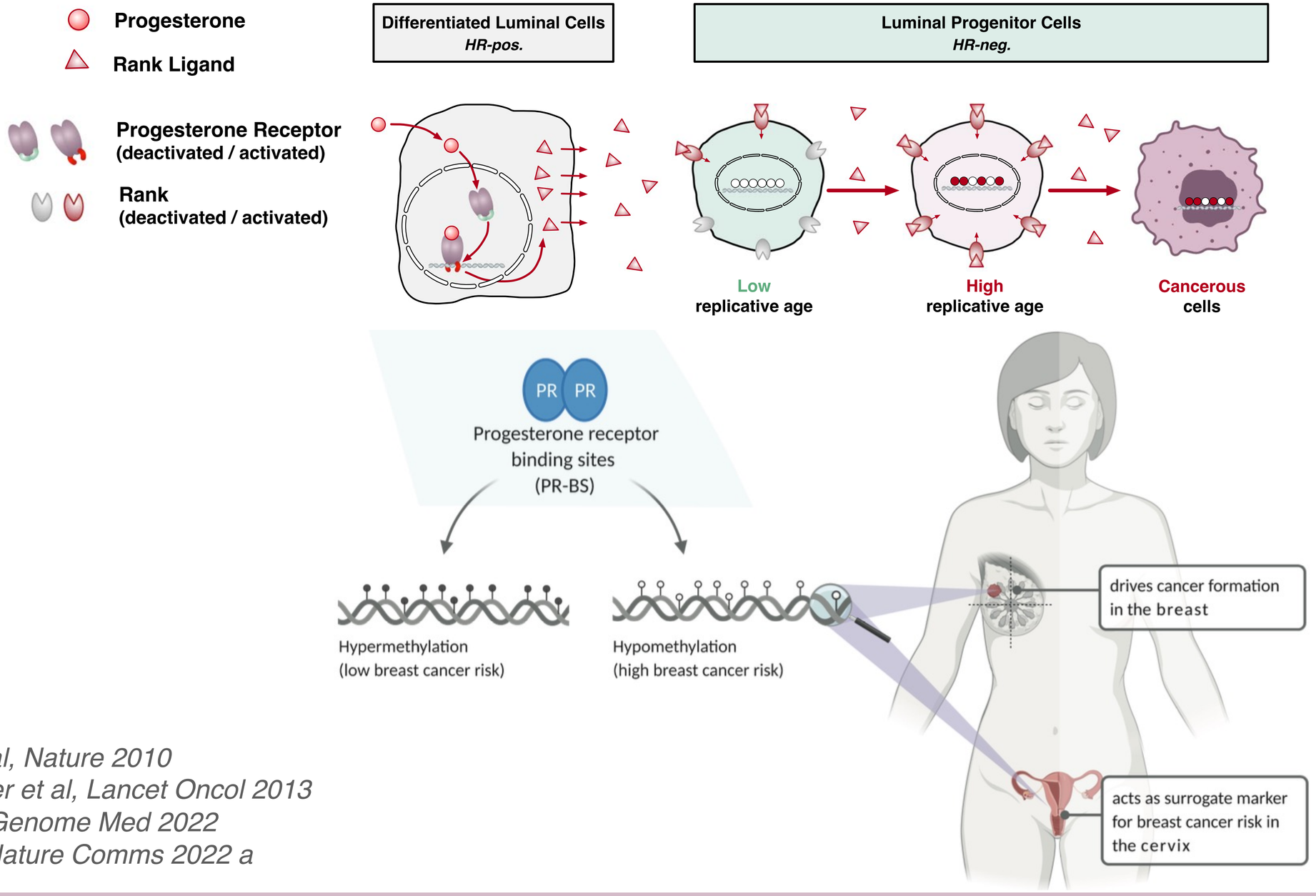
Surrogate tissue – ideally self-sampling is feasible

OmniSwab

ORAcollect®·Dx
OCD-100

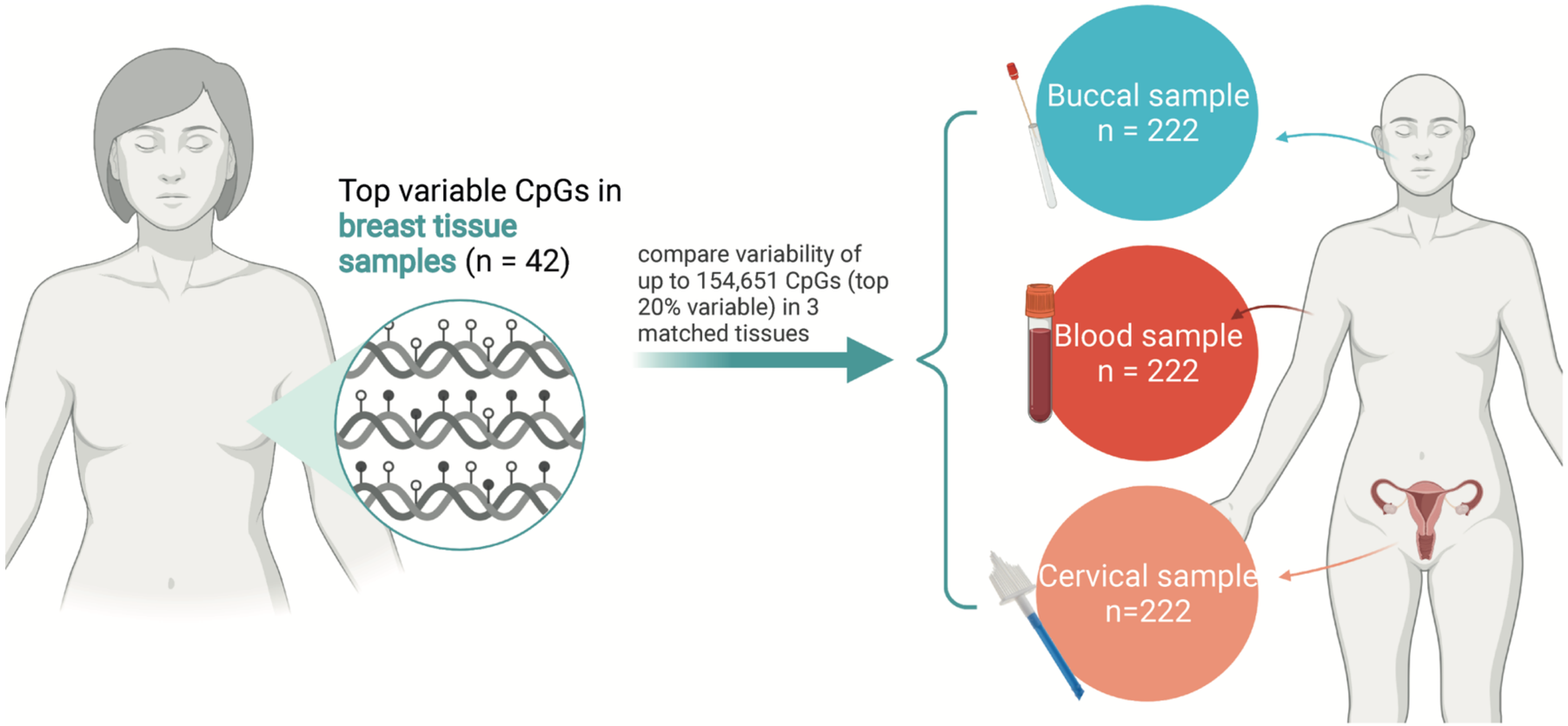


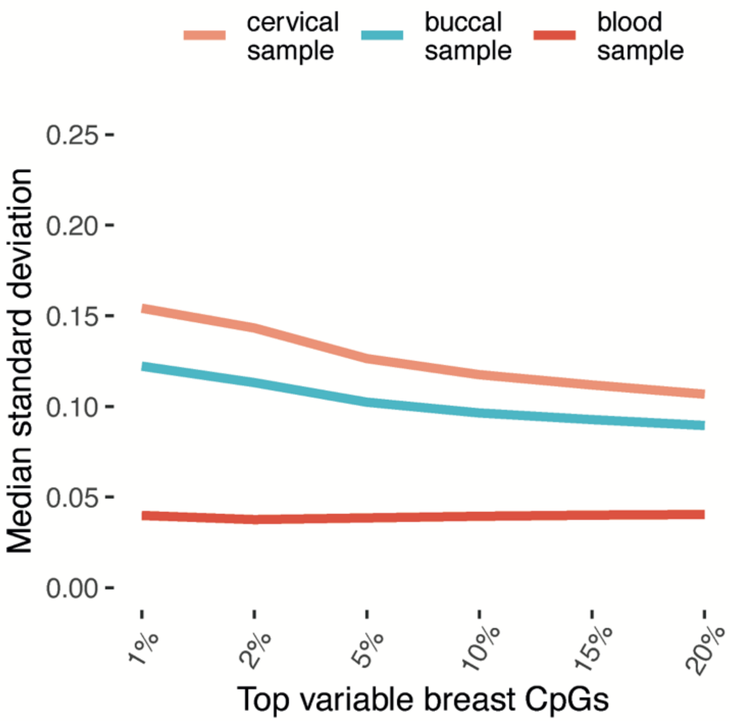
Surrogate tissue – capturing specific disease pathways



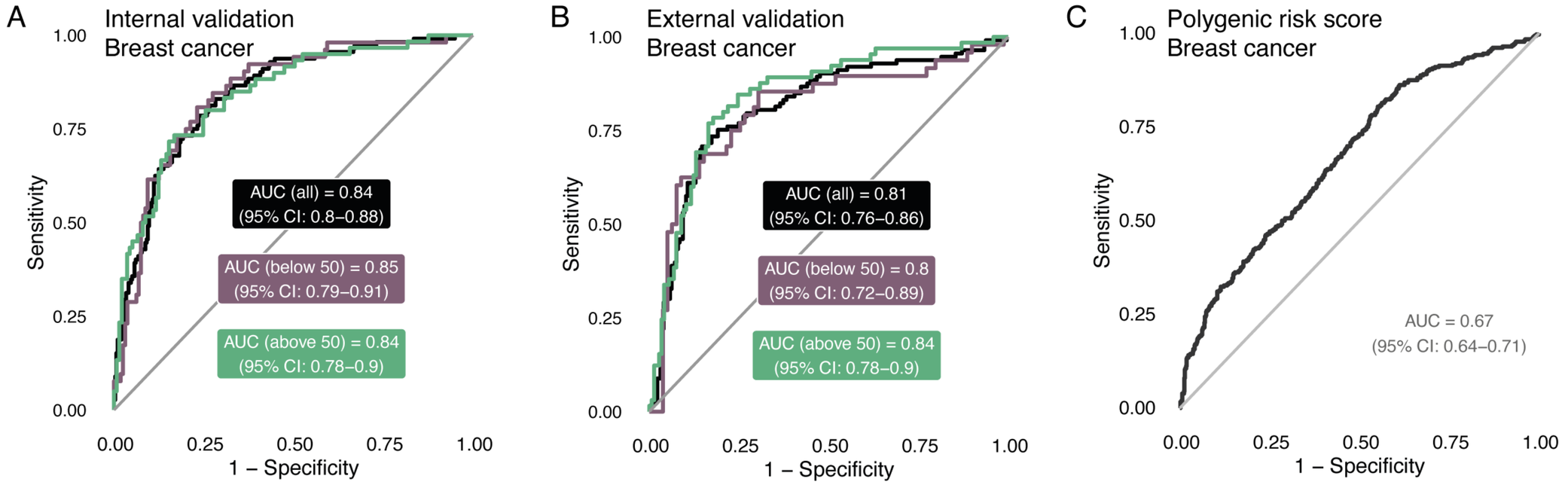
Schramek et al, Nature 2010
Widschwendter et al, Lancet Oncol 2013
Bartlett et al, Genome Med 2022
Barrett et al, Nature Comms 2022 a

Surrogate tissue – reflective of variability in tissue at risk





Breast Cancer risk prediction – WID-BC



D

Quantile	Control	Cancer	OR (unadjusted)	OR (adjusted)
Internal validation				
(-1.53, -0.58)	75	2	1.00 (reference)	1.00 (reference)
(-0.58, -0.28)	74	5	2.42 (0.48,19.25)	2.29 (0.45, 17.15)
(-0.28, 0.07)	74	17	8.01 (2.17,56.31)	8.47 (2.23, 55.81)
(0.07, 1.62)	74	88	41.11 (12.33,274.77)	41.73 (12.2, 262.62)
External validation				
(-1.53, -0.58)	58	8	1.00 (reference)	1.00 (reference)
(-0.58, -0.28)	69	8	0.84 (0.29,2.46)	0.89 (0.3, 2.67)
(-0.28, 0.07)	50	14	2 (0.78,5.46)	2.57 (0.95, 7.51)
(0.07, 1.62)	48	83	12.19 (5.62,29.86)	15.67 (6.59, 42.38)

E

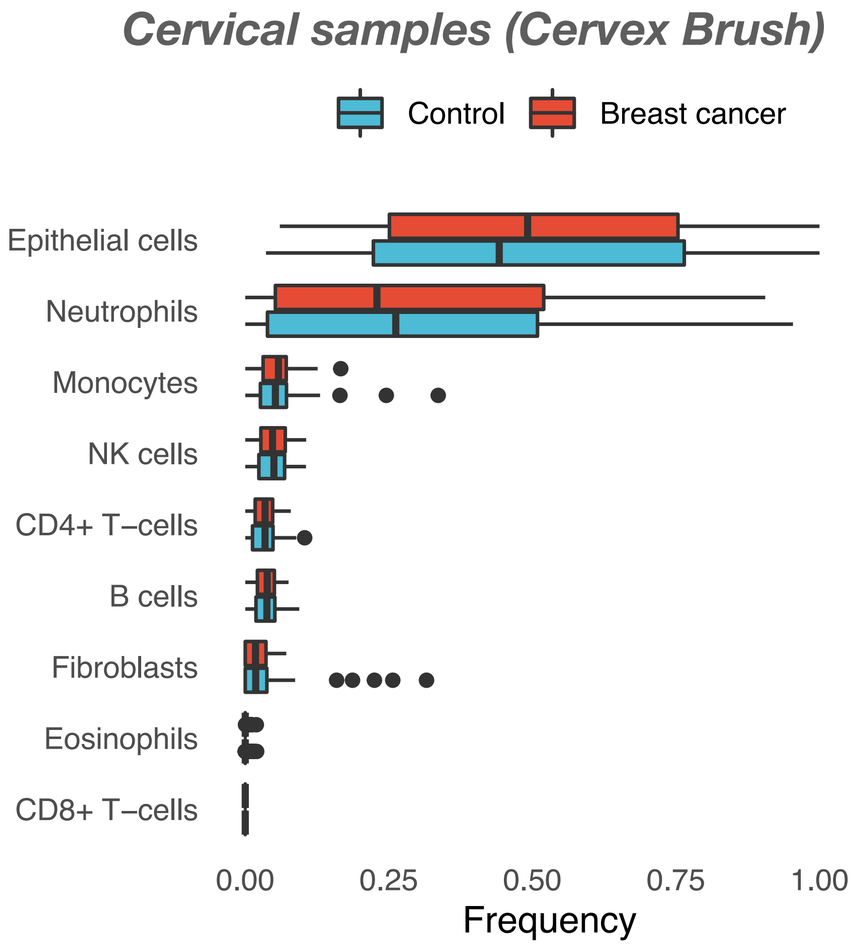
Risk group	Controls	Cases	OR (unadjusted)	OR (adjusted)
Internal validation				
low PRS_{313}, low WID™-BC	109	6	1.00 (reference)	-
high PRS_{313}, low WID™-BC	70	9	2.3 (0.78-7.3)	2.94 (0.95, 9.94)
low PRS_{313}, high WID™-BC	54	25	8.2 (3.3-23)	10.17 (3.68, 33.75)
high PRS_{313}, high WID™-BC	47	67	25 (11-69)	26.05 (11.15, 72.13)
External validation				
low PRS_{313}, low WID™-BC	82	9	1.00 (reference)	-
high PRS_{313}, low WID™-BC	57	13	2.1 (0.82-5.4)	2.26 (0.89, 5.96)
low PRS_{313}, high WID™-BC	41	29	6.3 (2.8-15)	10.59 (3.97, 32.71)
high PRS_{313}, high WID™-BC	30	60	18 (8.1-42)	18.35 (7.71, 49.24)

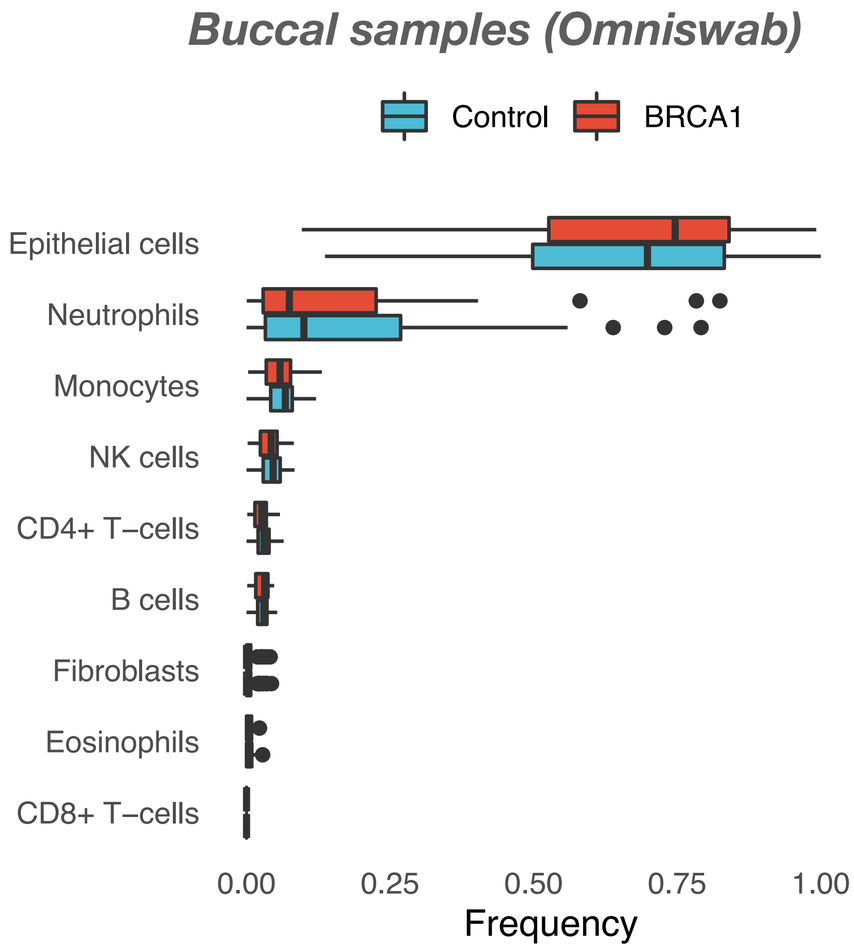
Barrett et al, Nature Comms 2022 a

Various issues to consider

- Tissue specificity
- Storage requirements
- Age dependence
- Hormonal (cycle) exposure
- Circadian rhythm

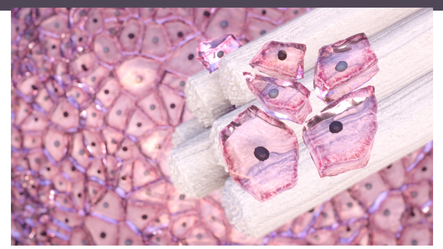
Assessment of proportion of cell types utilising DNAme in heterogenous samples





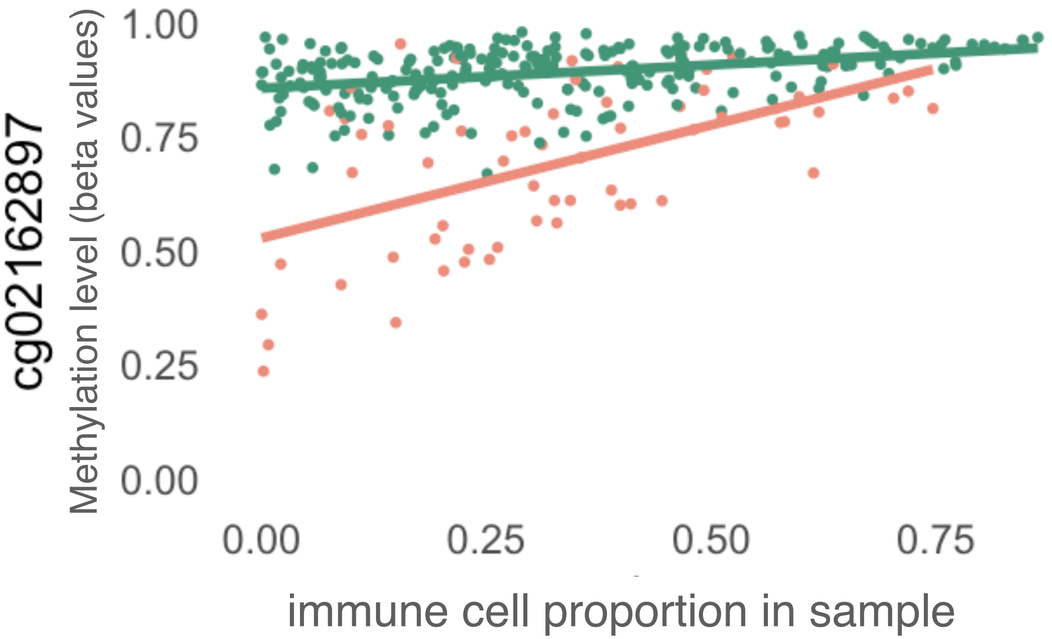
Barrett et al, Nature Comms 2022 a

Tissue dependent recording of environmental exposure

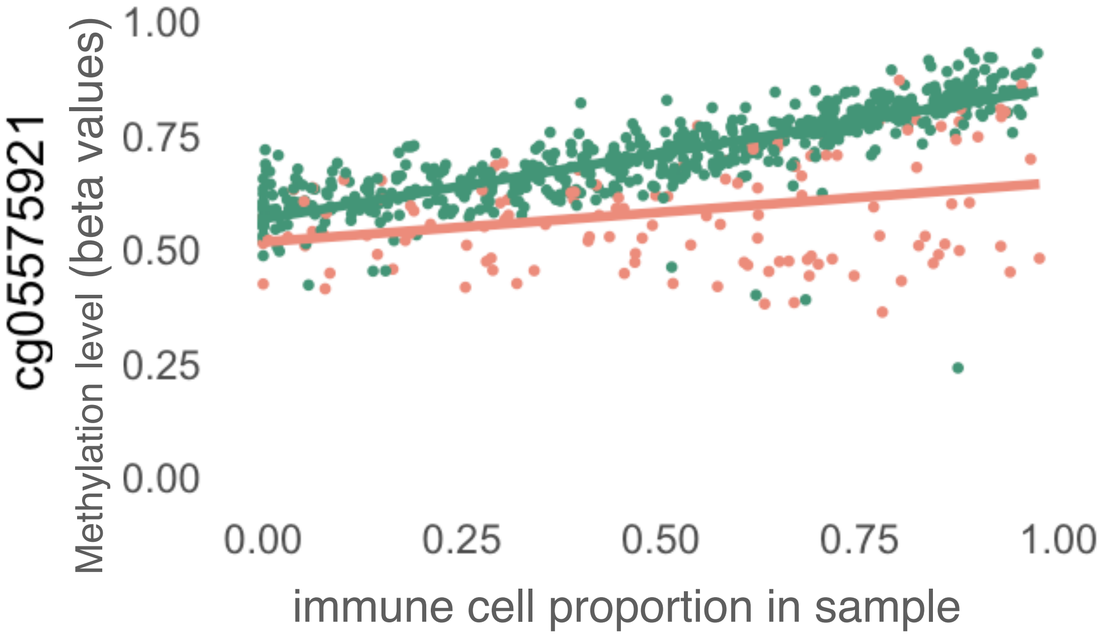


Control Smoker

Epithelial effect



Immune cell effect



unpublished

SUGGESTIONS for AUSTRIAN COHORTS

- Capturing factors determining health and disease
- Three different albeit complementary settings spanning the entire life-course
- Uniqueness (compared to existing international efforts)

  Longitudinal and repetitive sampling

  Self-sampling

  Including non-blood (i.e., epithelial)

Austrian Cohorts - Initiative 1: In utero exposure ("Austrian Birth Cohort")

- Only a few dedicated centres across Austria (E, W, S, N)
- Invitation of pregnant women (via office gynaecologists) who are likely to give birth in the relevant centres
- Collecting samples (blood, faeces, saliva, vaginal swab, buccal sample – assessing metaboloms/toxins, microbiomes, etc.) and data/questionnaires throughout pregnancy and documenting the course of pregnancy
- Collecting placental and umbilical blood at birth and isolate:
  - Placental cells (fibroblasts, endothelial cells, macrophages, trophoblastic cells)
  - CD34 cells from umbilical blood
- Readout from foetal/placental cells are:
  - DNAmut
  - DNAme
  - Other omics
  - In a subsetset: Organoids and possibly plasticity of cells (i.e., proportion of successful induction of pluripotency from placental fibroblasts, etc.)
- Link of exposure/data collected throughout pregnancy with the readout data
- Long-term follow up data of children

Challenges: *Standardised procedures, laborious and time-consuming procedures, expected relative low numbers*

Austrian Cohorts - Initiative 2: "Austrian's Women's Health Cohort"

- Women attending Mammography Screening Centres
- Dedicated App to obtain epidemiological data
- Link to registries
- Electronic consenting
- Collection of four specimens at the outset:
  - Cervical self-sample (Evalyn brush → Thinprep)
  - Healthcare professional sample (Cervex → Thinprep)
  - Buccal self-sample (HRC-100)
  - Urine
- Repeat collection (3 specimens) every year (approached by post, self-sampling only)
- Collection of epidemiological (volunteer-reported) every 6 months
- Access to imaging data (i.e., breast density)
- Initiative expanding to other European countries (SWE, IT)
- Core purpose is to develop/validate risk predictive algorithms

Challenges: *To standardise procedures, to secure buy in from screening centres*

Austrian Cohorts - Initiative 3: Population Age cohort ("Austrian Aging Cohort")

- Dedicated campaign in specific Austrian cities, villages, communities, companies
- Women and men > 18 years of age (no upper age limit)
- Providing buccal self-sample every 6 months (HRC-100; sent by post)
- Dedicated App to obtain epidemiological data (volunteer-reported) every 6 months
- Link to registries
- Electronic consenting
- Core purpose is to identify factors that accelerate or decelerate the ageing process (e.g. telomere lengths, DNAmut like CHIP, DNAme, etc) and to assess whether the slope of the ageing curve (i.e., as assessed by longitudinal/repetitive measurements) and its relation to chronological age is associated with disease risk (i.e., cancer, metabolic, cardiovascular, neurodegeneration)

Challenges: *To standardise procedures, to secure buy in from screening centres*

Austrian Cohorts - Bringing Initiatives 1, 2 and 3 together

Example:

Assess whether molecular data that indicate disease risk (e.g., for BC; evidence obtained in Initiative 2) are being initiated in utero by specific exposures (e.g., Bisphenol as assessed in Initiative 1) and whether molecular features which are driven by in-utero exposure are involved in the ageing process (Initiative 3)

DISCUSSION