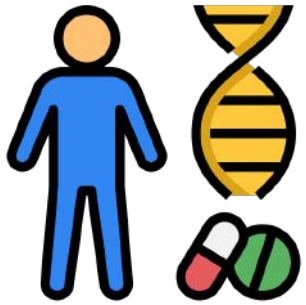


Genomic Testing in Cancer

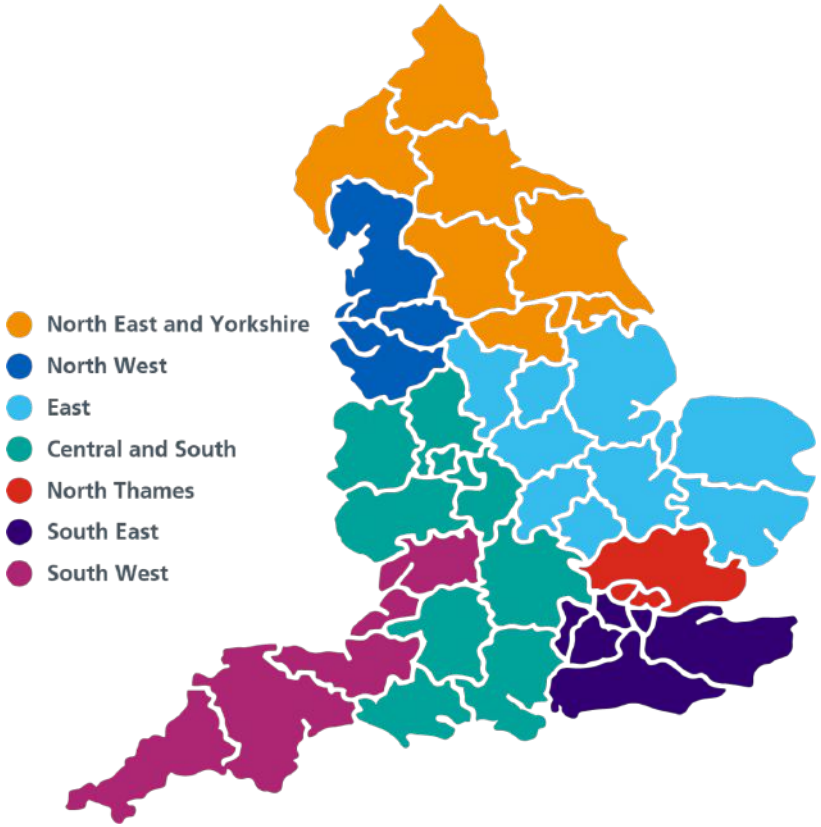
Chris Watt

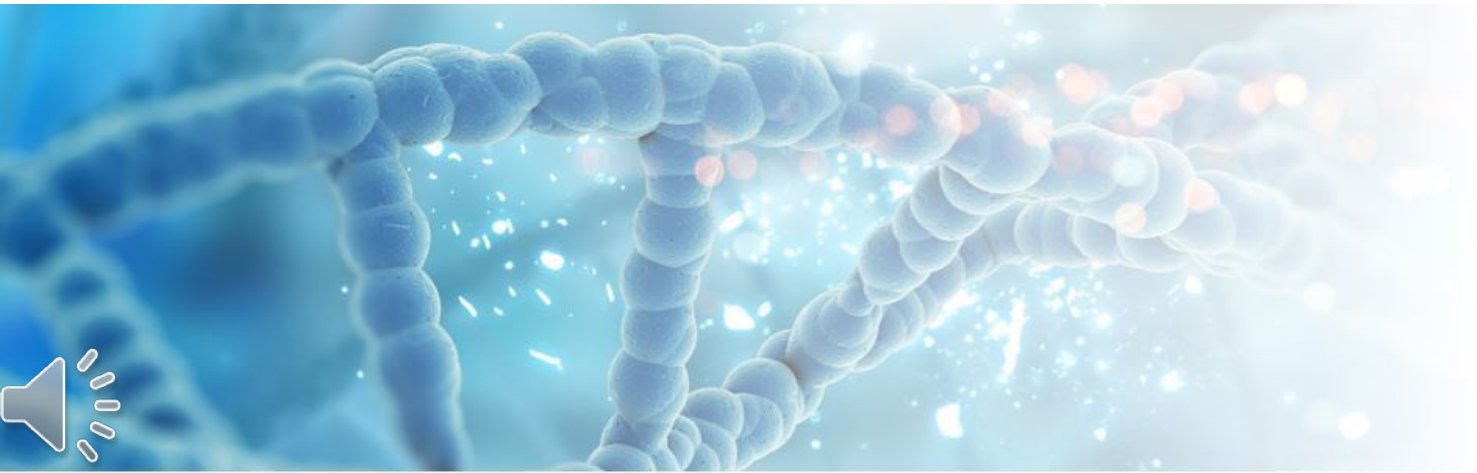
Principal Clinical Scientist & Genomic Educator

NHS
North West
NHS Genomic Laboratory Hub

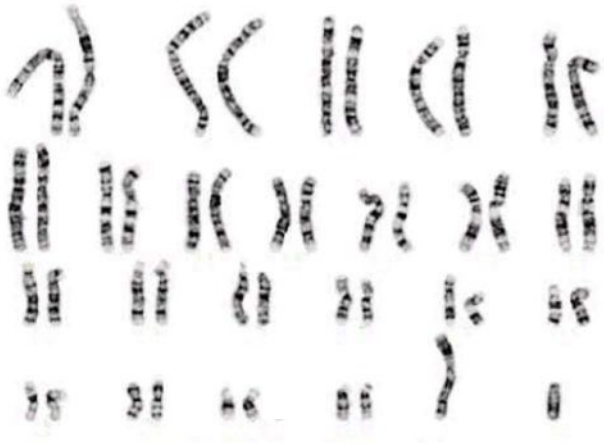
St Mary’s Hospital, Manchester

Liverpool Women’s Hospital



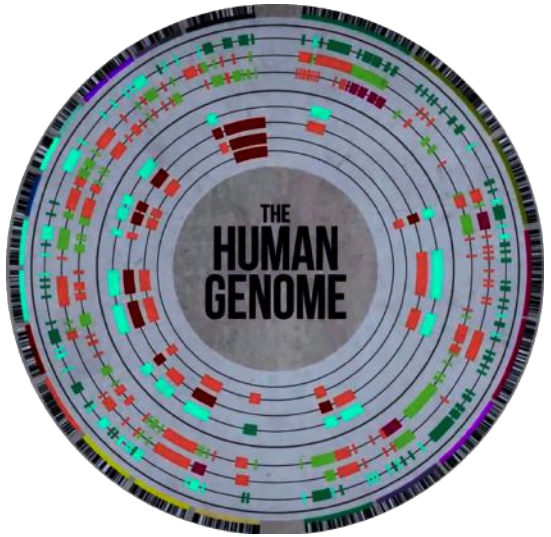


NHS
North West
NHS Genomic Laboratory Hub



human karyotype

(Re)Introducing…
The Human Genome

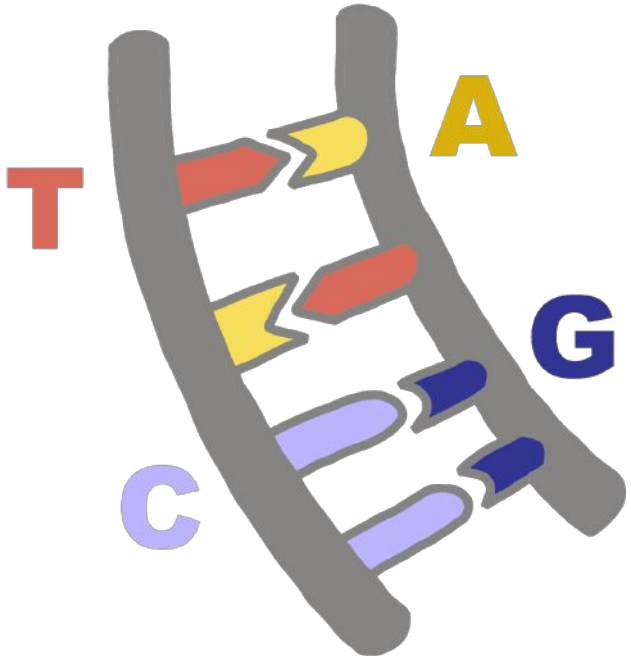


chromosome

gene

DNA

protein



Cancer is a disease of the genome

TGCCTA UNMUTATED DNA

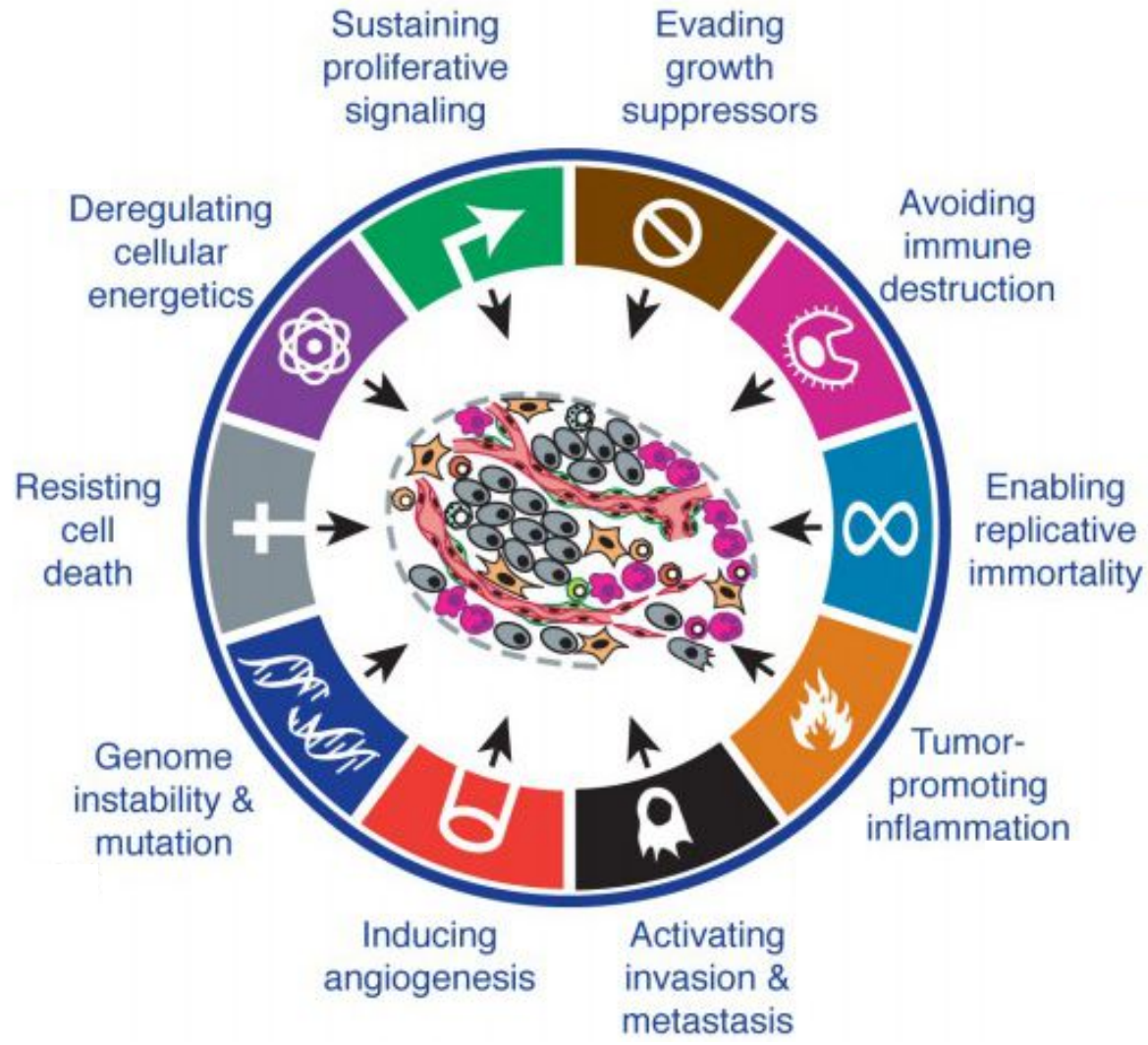
TGACTA SUBSTITUTION

TGCCCTA INSERTION

TGCTA DELETION

TGCATC INVERSION

The Hallmarks of Cancer:



Ref: Hallmarks of cancer: the next generation (https://pubmed.ncbi.nlm.nih.gov/21376230/)

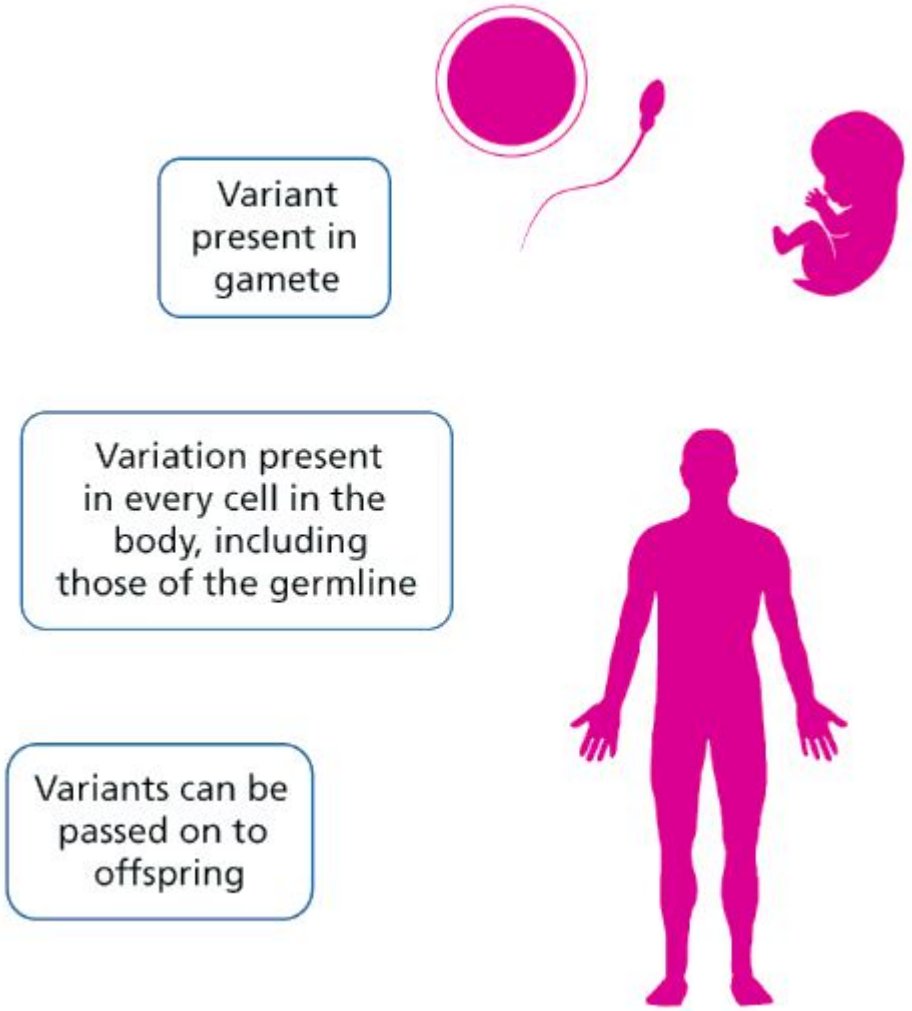
Germline variants vs Somatic variants

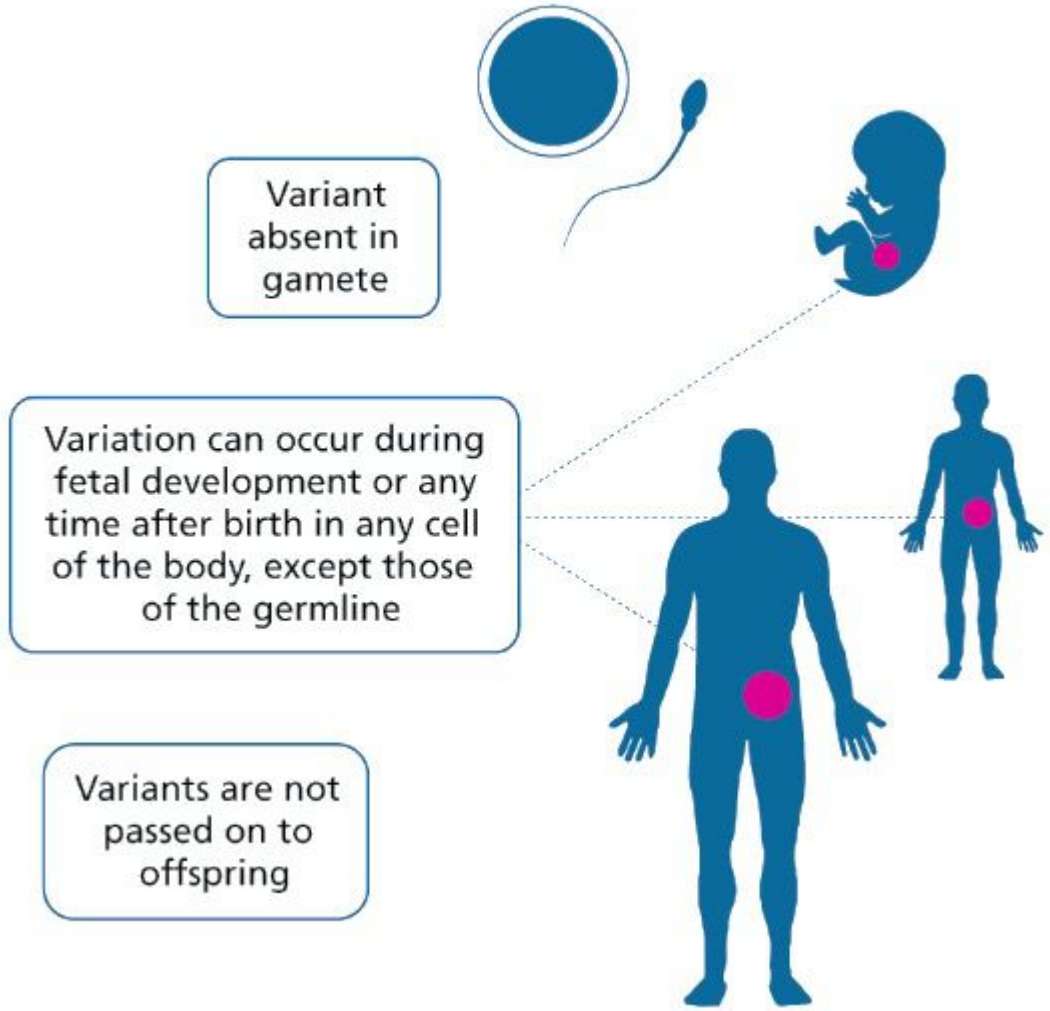
Variant present in gamete

Variation present in every cell in the body, including those of the germline

Variants can be passed on to offspring

Germline variants vs Somatic variants

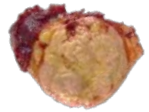




Germline variant testing vs Somatic variant testing

Sample type: **Blood**

Sample type: **Tumour tissue**

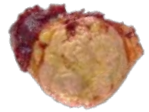


Clinical Utility	Clinical Utility

Germline variant testing vs Somatic variant testing

Sample type: **Blood**

Sample type: **Tumour tissue**



Clinical Utility

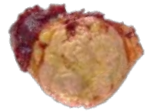
- Confirm presence or absence of germline variant, provides answers to the diagnosis
- Determine risk of developing a cancer associated with germline variant detected in family member
- Access to preventative treatment, prophylactic surgery or screening
- Determine risk of passing on cancer risk to offspring
- Useful information for family planning

Clinical Utility

Germline variant testing vs Somatic variant testing

Sample type: **Blood**

Sample type: **Tumour tissue**



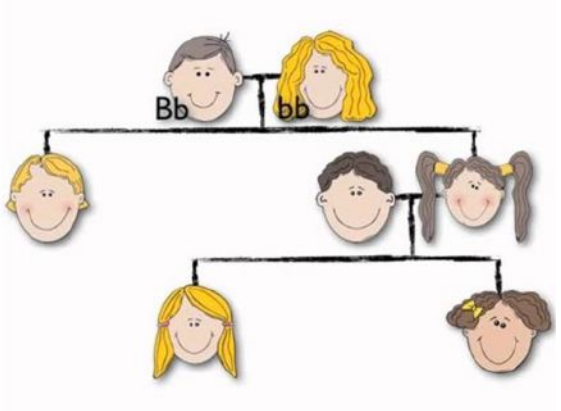
Clinical Utility

- Confirm presence or absence of germline variant, provides answers to the diagnosis
- Determine risk of developing a cancer associated with germline variant detected in family member
- Access to preventative treatment, prophylactic surgery or screening
- Determine risk of passing on cancer risk to offspring
- Useful information for family planning

Clinical Utility

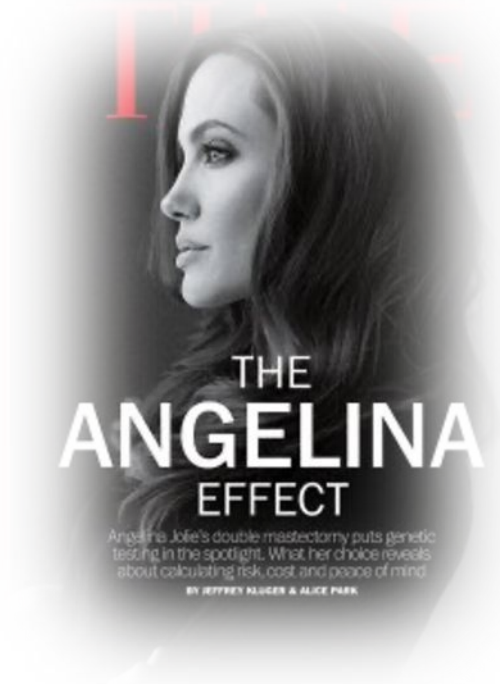
- To help confirm a particular diagnosis
- To provide useful and accurate prognostic information
- To provide targeted treatment options
- To identify germline variants

Germline variant testing

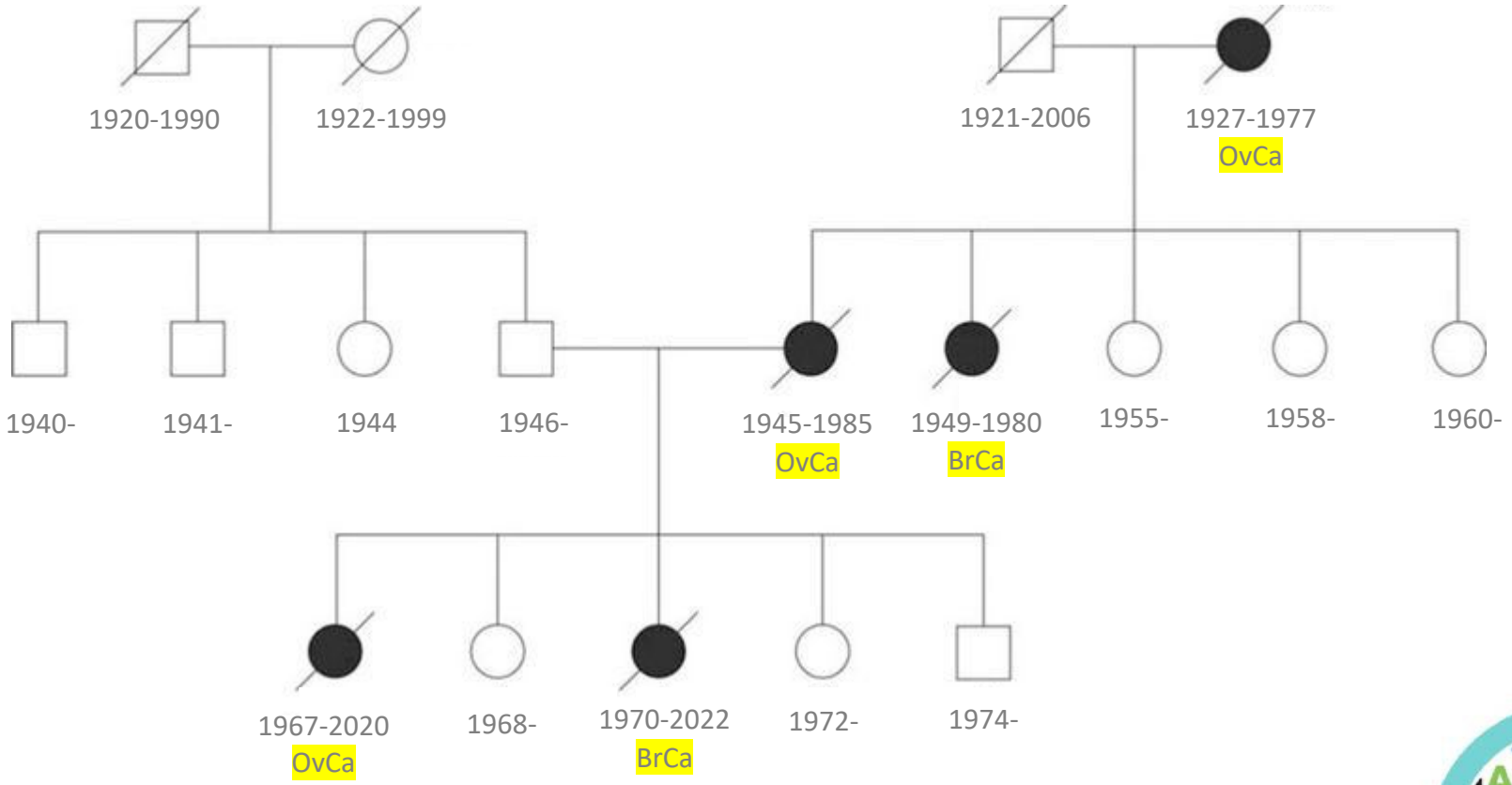


- Hereditary Breast and Ovarian Cancer (HBOC)
  - ***BRCA1*** and ***BRCA2*** variants
  - Up to 85%-90% risk of developing **breast** cancer
  - Up to 40-60% risk of developing **ovarian** cancer
  - Increased risk of prostate, skin and pancreatic cancers
  - Accounts for ~15% of all breast cancer cases
- Lynch syndrome
  - Variants in DNA repair genes (***MLH1***, ***MSH2***, ***MSH6*** and ***PMS2***)
  - Increased risk of **colon** cancer (75% risk), **endometrial** cancer (50%), **prostate** cancer (20%), **breast** cancer (18%), and **other** tumour types
  - Accounts for ~3% of all colon cancer cases

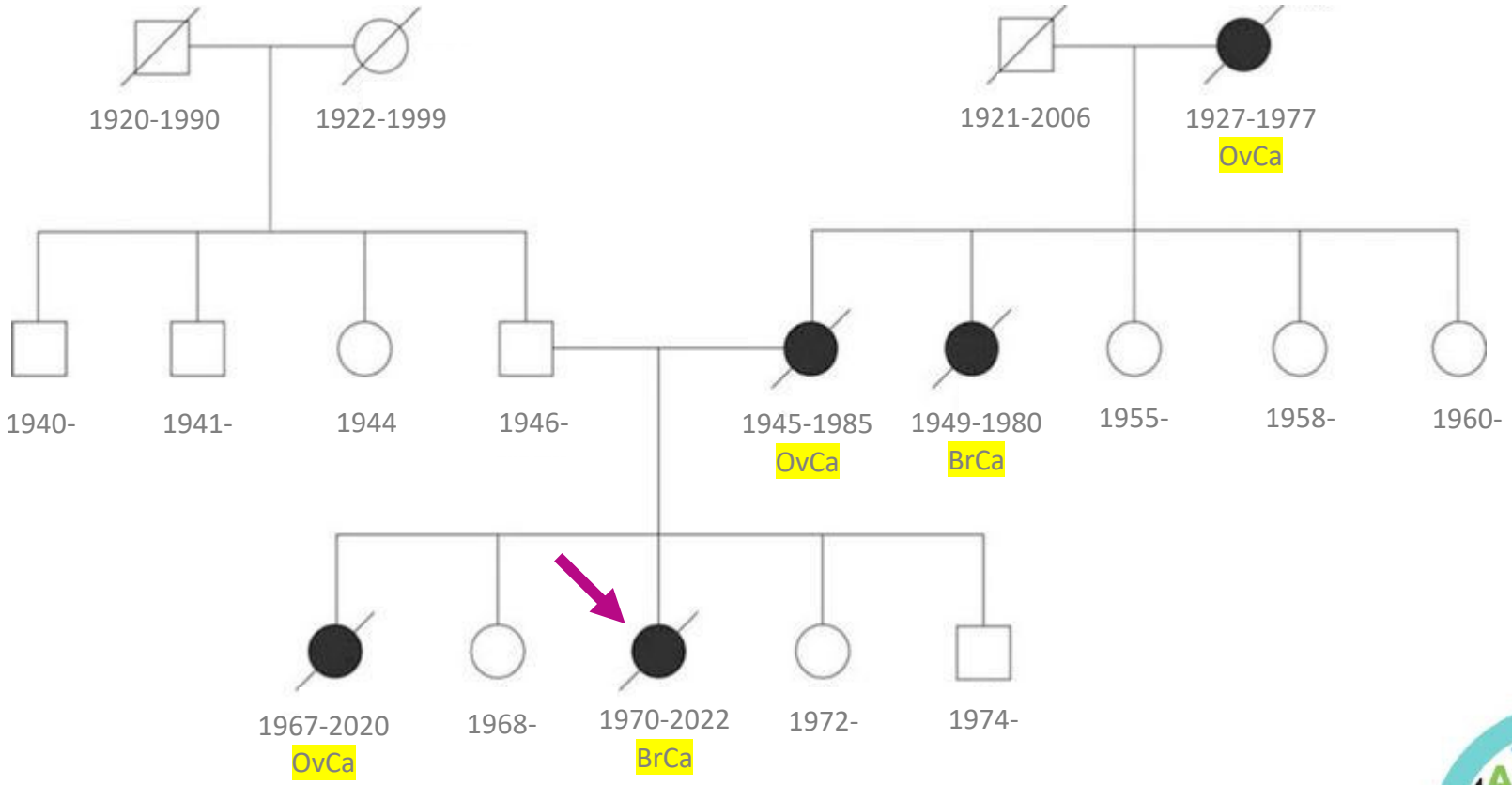
Germline variant testing



Germline variant testing - Family case study

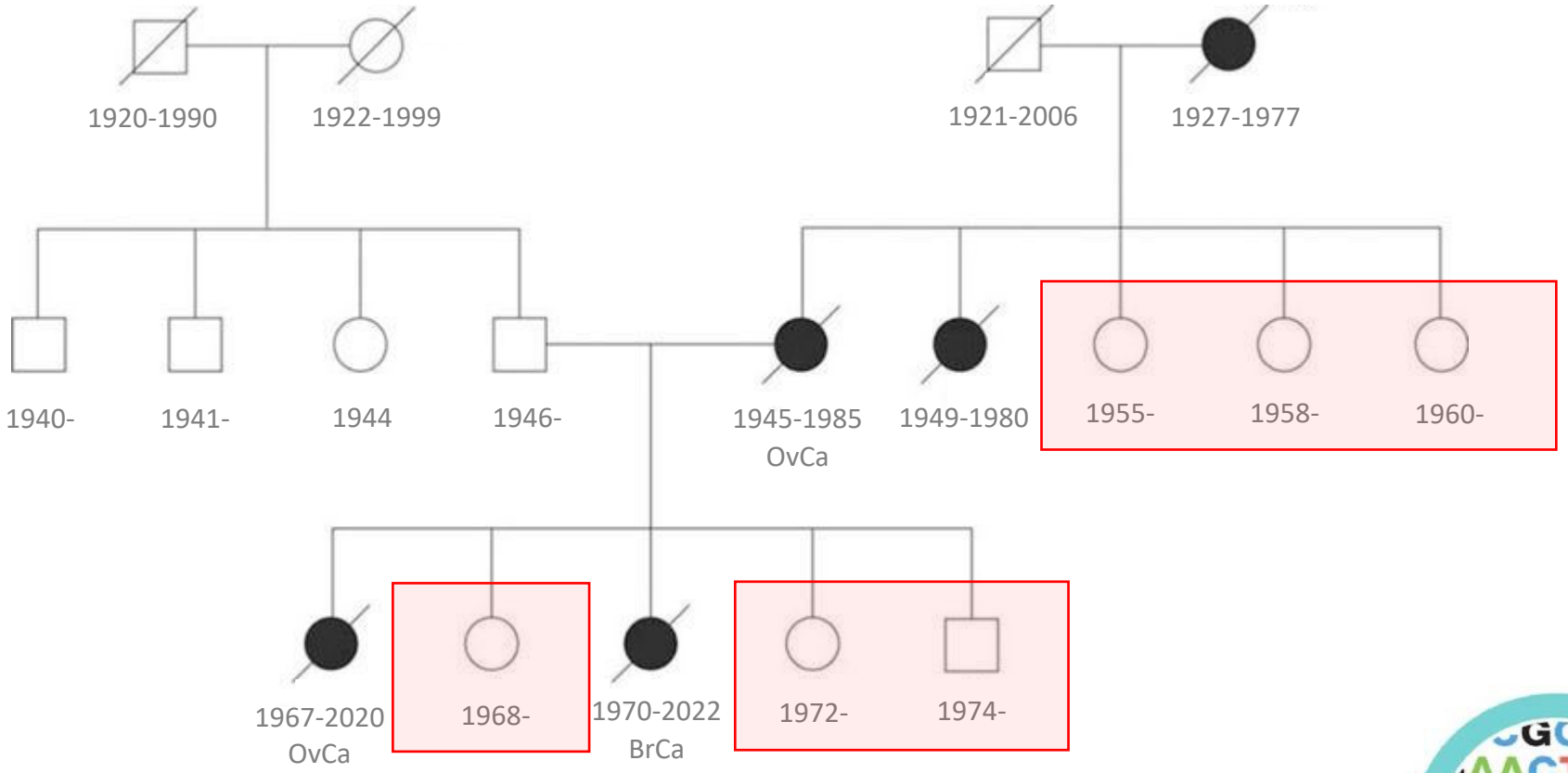


Germline variant testing - Family case study



BRCA1 c.505CT p.(Gln169Ter)

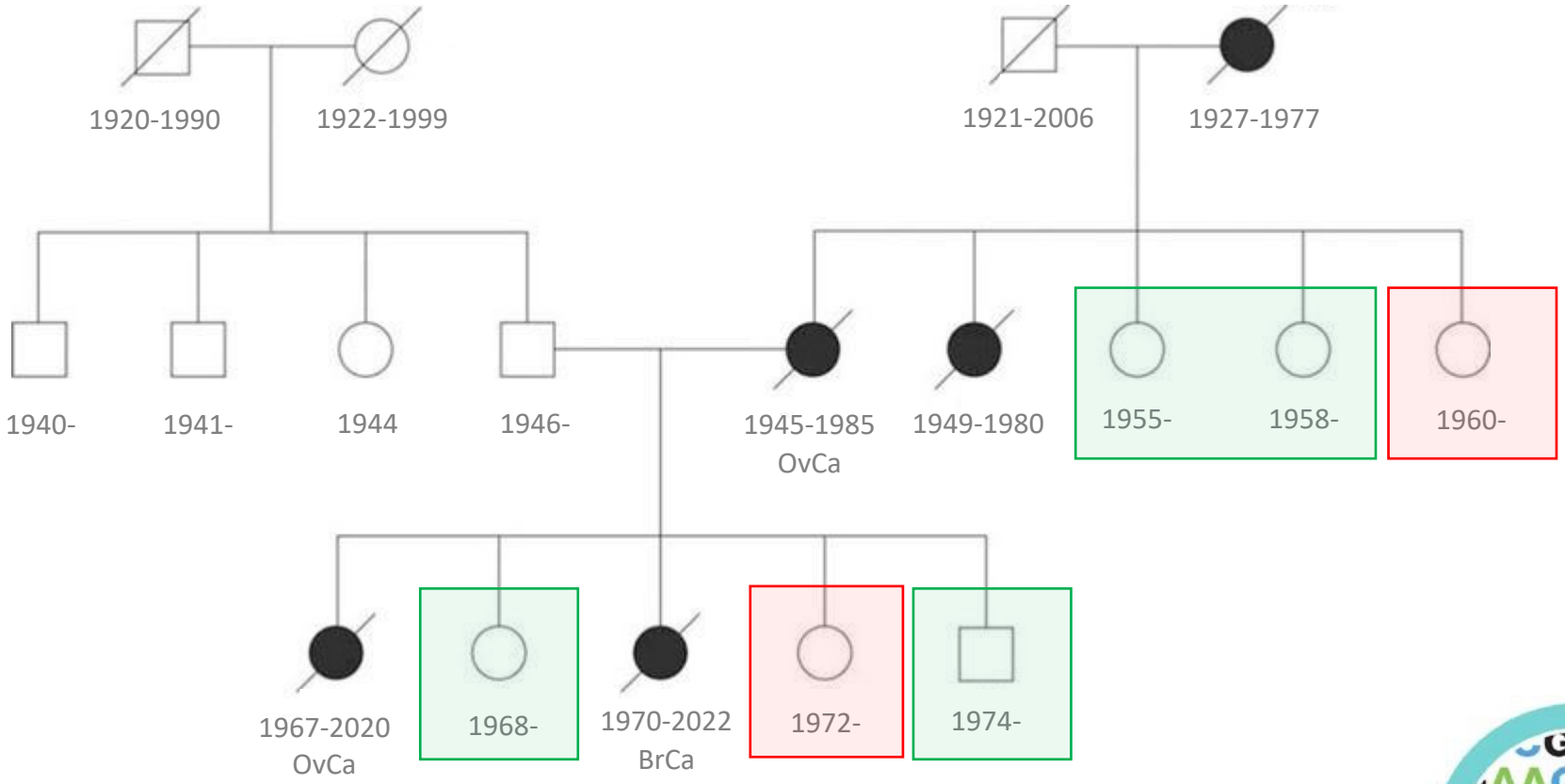
Germline variant testing - Family case study



50% risk of carrying the germline variant = Stress, regular screening, ?risk-reducing surgery ...

Germline variant testing

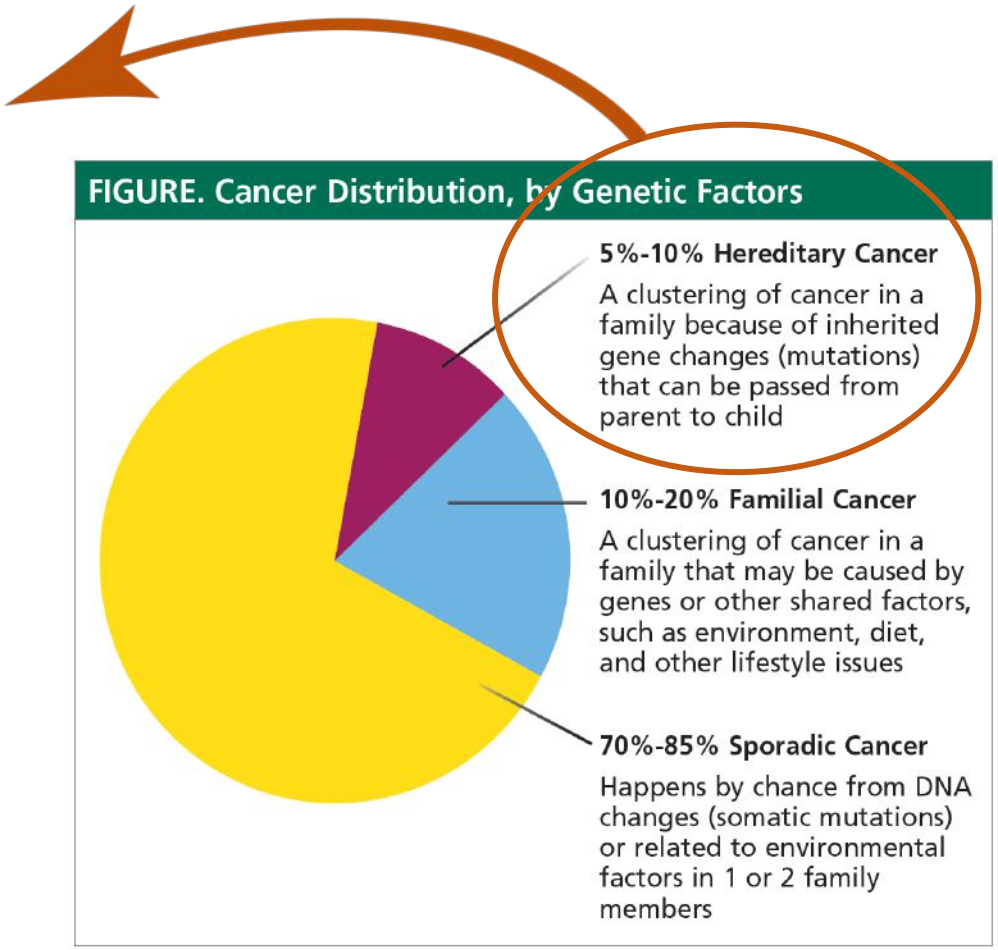
Variant **absent**… General population risk, less stress, no further screening or preventative surgery



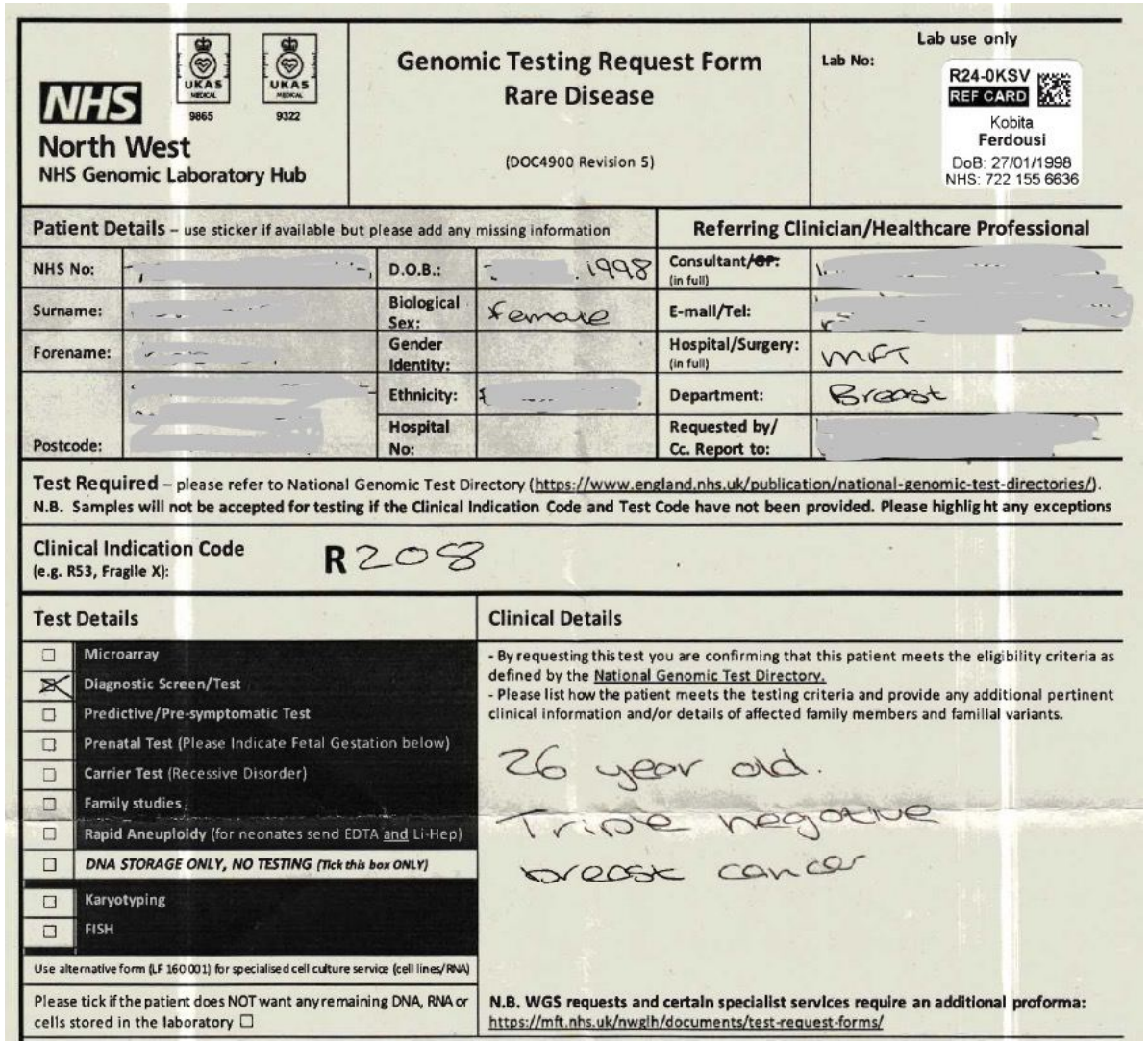
Variant **present**… Stress, regular screening, ?mastectomy/oophorectomy...

Inherited cancer testing: All services

Service	Genes	Clinical utility
Inherited Cancer NGS Panel	Genes associated with: Lynch syndrome, breast & ovarian cancer, other cancer syndromes	Germline testing of cancer-predisposition genes in **affected** or **at-risk individuals** Detection of clinically significant variants very important for clinical management of patient and family members
Cascade Testing (Predictives & Confirmations)	Gene/variant of interest	Targeted testing of **specific familial variants** to determine whether an individual has inherited the variant previously detected in their family member.
Deceased Index Testing (of tumour tissue)	Genes associated with: Lynch syndrome, breast & ovarian cancer, other cancer syndromes	Offered for families **with no living affected relatives** but there is pathology tissue available. This can be very beneficial in the management of asymptomatic family members to determine their risk status and whether they may need germline testing.
RB1 & NF1	RB1 & NF1	Germline testing of retinoblastoma and neurofibromatosis patients for the detection of causative variants in the RB1 and NF1 genes



Inherited cancer testing: Case study



NHS North West
NHS Genomic Laboratory Hub

UKAS MEDICAL 9865 UKAS MEDICAL 9322

Genomic Testing Request Form
Rare Disease

(DOC4900 Revision 5)

Lab use only
Lab No: R24-0KSV REF CARD
Kobita Ferdousi
DoB: 27/01/1998
NHS: 722 155 6636

Patient Details – use sticker if available but please add any missing information

NHS No:	[redacted]	D.O.B.:	[redacted] 1998	Consultant/~~GP~~: (in full)	[redacted]
Surname:	[redacted]	Biological Sex:	Female	E-mail/Tel:	[redacted]
Forename:	[redacted]	Gender Identity:		Hospital/Surgery: (in full)	MFT
Postcode:	[redacted]	Ethnicity:	[redacted]	Department:	Breast
		Hospital No:		Requested by/ Cc. Report to:	[redacted]

Referring Clinician/Healthcare Professional

Test Required – please refer to National Genomic Test Directory (https://www.england.nhs.uk/publication/national-genomic-test-directories/).
N.B. Samples will not be accepted for testing if the Clinical Indication Code and Test Code have not been provided. Please highlight any exceptions

Clinical Indication Code (e.g. R53, Fragile X): R208

Test Details	
☐	Microarray
☒	Diagnostic Screen/Test
☐	Predictive/Pre-symptomatic Test
☐	Prenatal Test (Please Indicate Fetal Gestation below)
☐	Carrier Test (Recessive Disorder)
☐	Family studies
☐	Rapid Aneuploidy (for neonates send EDTA and Li-Hep)
☐	***DNA STORAGE ONLY, NO TESTING (Tick this box ONLY)***
☐	Karyotyping
☐	FISH

Use alternative form (LF 160 001) for specialised cell culture service (cell lines/RNA)

Please tick if the patient does NOT want any remaining DNA, RNA or cells stored in the laboratory ☐

Clinical Details

- By requesting this test you are confirming that this patient meets the eligibility criteria as defined by the National Genomic Test Directory.
- Please list how the patient meets the testing criteria and provide any additional pertinent clinical information and/or details of affected family members and familial variants.

26 year old.
Triple negative
breast cancer

N.B. WGS requests and certain specialist services require an additional proforma:
https://mft.nhs.uk/nwglh/documents/test-request-forms/

- 26-year-old female diagnosed with triple negative breast cancer
- Qualifies for **BRCA1 & BRCA2** germline variant testing due to young age and type of breast cancer
- BRCA1/BRCA2 germline variants most common in the ‘triple negative’ breast cancer subtype

Inherited cancer testing: Case study

NGS panel testing:

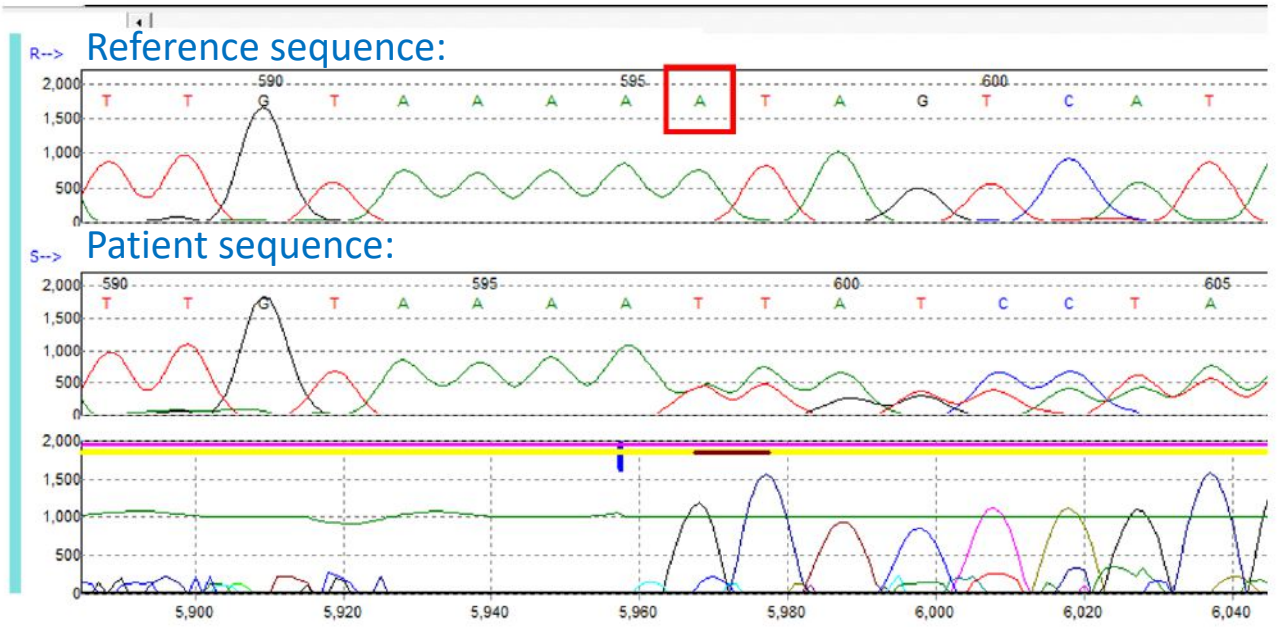
Chr	St	End	Gene	Transcript	HGVS_cDNA	HGVS_protein	RunFreq	Depth	Ratio(%)	Mean_Ru	Allele1_C	Allele2_C	Allele1_M	Allele2_M	QUAL	Is
13	32893198	32893198	675;BRCA2	NM_000059.3	c.68-16delT	-	7/8	1113	2.07	1.93	1087	23	35	34	58	N
13	32911736	32911736	675;BRCA2	NM_000059.3	c.3244delA	p.(Asn1083IlefsTer4)	1/8	2266	48.12	-	1175	1090	35	34	255	P

CanVIG-UK Cancer Variant Interpretation Group UK

CanVIG-UK Consensus Specification for Cancer Susceptibility Genes (CSGs) of ACGS Best Practice Guidelines for Variant Classification

= classified as **PATHOGENIC**

Variant confirmed by Sanger sequencing:



Inherited cancer testing: Case study

REASON FOR REFERRAL: Diagnostic. Locally advanced breast cancer. Triple negative.

RESULT SUMMARY:
Pathogenic variant detected in BRCA2
Genetic diagnosis of BRCA2 associated cancer susceptibility

RESULT AND INTERPRETATION:

1. This patient is heterozygous for a pathogenic BRCA2 variant c.3248del p.(Asn1083IlefsTer4) (details in Appendix II overleaf) in their lymphocyte DNA. Monoallelic pathogenic BRCA2 variants cause cancer susceptibility (OMIM #612555), particularly breast and ovarian cancer in females.

2. This patient is at increased risk of developing further BRCA2 associated cancers and should be managed appropriately.

3. The presence of an inherited BRCA2 pathogenic variant increases the likelihood of a response to PARP inhibitor therapy. Please refer to the current NICE guidance regarding PARP inhibitor therapy
Breast cancer: https://www.nice.org.uk/guidance/ta886

4. This result has implications for other family members. Testing for this variant is available to other relatives of this patient, as appropriate (via referral to a clinical genetics service).

Please see page 2 for appendix.

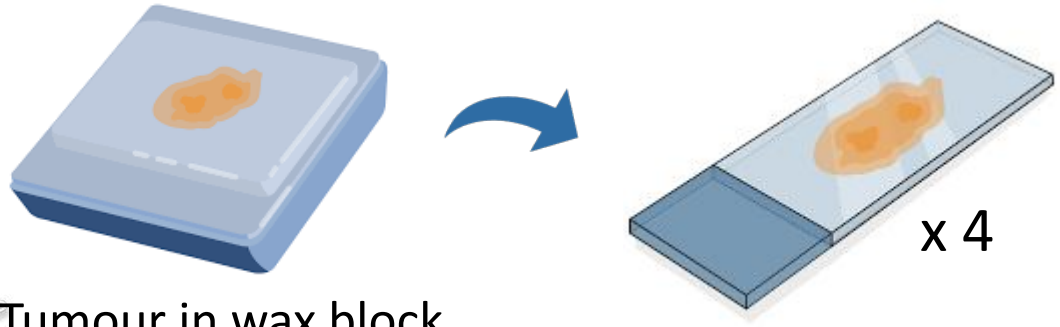
Somatic cancer services

Treatment Diagnostic Prognostic

Service	Gene targets	Clinical utility
Lung cancer	EGFR KRAS BRAF MET	Patients with driver variants are eligible for EGFR-inhibitors (e.g. gefitinib) Patients with the KRAS G12C variant are eligible for the KRAS inhibitor sotorasib Patients with driver variants are eligible for clinical trials (BRAF/MEK inhibitors e.g. dabrafenib) Patients with an exon 14 skipping variant are eligible for MET inhibitors (e.g. capmatinib)
Ovarian cancer	BRCA1/BRCA2 HRD	Patients with BRCA1/2-mutant or HRD positive tumours are eligible for PARP inhibition therapy (e.g. olaparib)
Colorectal cancer	KRAS/NRAS BRAF PIK3CA	Patients with KRAS/NRAS drivers predicted to be resistant to anti-EGFR monoclonal antibodies Patients with BRAF drivers may benefit from doublet/triplet therapy regimens Patients with PIK3CA drivers may be eligible for clinical trials (e.g. P13K/AKT pathway inhibitors
Melanoma	BRAF NRAS KIT	Patients with BRAF V600 driver variants eligible for BRAF/MEK inhibitor therapy (e.g. vemurafenib/trametinib) Patients with NRAS driver variants have a poorer prognosis Patients with KIT driver variants may be eligible for imatinib therapy or other clinical trials
Brain tumours	IDH1/IDH2 1p19q co-deletion KIAA1549::BRAF fusion RELA fusion	Glioma patients who are IDH1/2 wildtype – consistent with diagnosis of grade IV glioblastoma Glioma patients with 1p19q co-deletion – consistent with diagnosis of oligodendroglioma Glioma patient with KIAA1549::BRAF fusion – consistent with diagnosis of pilocytic astrocytoma Glioma patient with a RELA fusion – consistent with diagnosis of supratentorial ependymoma
GIST	KIT PDGFRA	Oncogenic drivers in KIT or PDGFRA confirm a molecular diagnosis of gastrointestinal stromal tumour (GIST) Also provides information on treatment options (e.g. imatinib) and likelihood of response/resistance
MLH1 promoter hypermethylation	MLH1 promoter	As part of the Lynch Syndrome screening pathway; MLH1 promoter hypermethylation increases likelihood of CRC being somatic in origin therefore not referred for Lynch syndrome germline screening
Microsatellite instability (MSI)	5 repeat markers	As part of the Lynch Syndrome screening pathway; MLH1 promoter hypermethylation increases likelihood of CRC being somatic in origin therefore not referred for Lynch syndrome germline screening MSI-high used as treatment biomarker for immunotherapy (e.g. pembrolizumab) in certain tumour types
Fusion panel (all cancer types)	Oncogene panel Sarcoma panel	Patients with oncogenic fusions involving genes such as ALK, ROS1, RET, NTRK are eligible for gene specific inhibitors (e.g. crizotinib, entrectinib, selpercatinib) Oncogenic fusions may confirm a diagnosis of a specific sarcoma (e.g. NAB2-STAT6 is diagnostic of solitary fibrous tumour)

Somatic cancer testing: Case study

- 43-year-old woman recently diagnosed with stage 4 lung cancer (non-smoker)
- Lung biopsy -> tumour embedded into wax -> mounted on to slides
- Four tumour slides from a right upper lung biopsy have been sent for Lung Cancer NGS panel testing to determine targeted treatment options



Tumour in wax block

NHS

Lung Cancer Tumour Test Request Form

UKAS MEDICAL 9065

Patient Details	Referring Clinician
Surname	
Forenam	
DoB: 2	
Sex:	

CLINICAL DETAILS:

Stage 4 Lung cancer (NSCLC)
Non-smoker. lung biopsy.

PLEASE INCLUDE A COPY OF THE PATHOLOGY REPORT

Pathology block/sample no.:

Sampling Date:

CI Code*	Clinical Indication Name	Test Name	Test Code	Please tick
M4	Non-Small Cell Lung Cancer	EGFR, BRAF, KRAS, MET	M4.1	✓
		ROS1, RET, ALK, NTRK fusions	M4.2	
		Urgent EGFR targeted testing##	M4.4	
		ctDNA #	M4.5	
		ALK/ROS1 FISH (*delete as appropriate*)	M4.10/ M4.6	
M231	Small cell lung cancer	RB1	M231.1	
		NTRK fusions	M231.2	
M5	Mesothelioma	NTRK fusion	M5.2	
		CDKN2A copy number	M5.3	
Various	Any Tumour Type	NTRK fusions	Various	

PATHOLOGY LABORATORY:

Please circle the approximate neoplastic cells (%) in the sample sent for analysis *(important in reducing risk of false negative results).*

1-5#	6-10#	11-20#
20-50 ✓	50-75	>75

Neoplastic cells in marked area _____%

#Where overall neoplastic cell content <20% and macrodissection would enhance % of neoplastic cells, please send slide mounted sections with corresponding marked H&E stained slide.

Somatic cancer testing: Case study

Tumour tissue received

DNA extracted

Pre-sequencing laboratory work

Sequencing

	A	B	C	D	H	K	L	M
1	**Chr**	**GRCh38 Region**	**Transcript**	**Variant_Nomenclature**	**Type**	**Variant Reads**	**Total Reads**	**Frequency (%)**
2	7	55191822	NM_005228.5	EGFR c.2573T>G p.(Leu858Arg) 28%	SNV	916	3250	28.18

Base affected

Amino acid change: Leucine to Arginine at amino acid 858 of EGFR

E G F R c . 2 5 7 3 T > G p . (L e u 8 5 8 A r g)

Gene name

Base change

Variant Interpretation

Result: **EGFR c.2573T>C p.(Leu858Arg)**

- Variant type
- Population data
- Cancer databases
- Functional studies
- In silico evidence

Pathogenic
Likely Pathogenic
Variant of Uncertain Significance (VUS)
Likely Benign
Benign

gnomAD
Genome Aggregation Database

COSMIC
Catalogue Of Somatic Mutations In Cancer

PubMed

DECIPHER
GRCh38

LUNG CANCER NGS PANEL TESTING REPORT

NAME:
DATE OF BIRTH:
SEX:
NHS No:

POSTCODE:
YOUR REF:
OUR LAB REF:
DATE:

REASON FOR REFERRAL: This patient has been diagnosed with adenocarcinoma of the lung. Sections derived from a right upper lung biopsy stated to have 20-50% neoplastic cell content have been sent for Lung Cancer NGS panel testing to help guide clinical management.

RESULTS:

NAME (DoB)	PATH LAB REF NO. (SAMPLE TYPE)	RESULT
		BRAF - NO VARIANT IDENTIFIED **EGFR c.2573T>G p.(Leu858Arg)** KRAS - NO VARIANT IDENTIFIED MET - NO VARIANT IDENTIFIED

RESULTS AND INTERPRETATION:

The EGFR c.2573T>G p.(Leu858Arg) variant was present in this patient's pathology sample (approximate variant allele frequency was 28% of reads). This is an activating variant previously described in tumours showing sensitivity to tyrosine kinase inhibitors (TKI).

No variants were detected in the BRAF, KRAS or MET genes.

In conclusion, the pathology sample tested exhibits a sensitising variant within EGFR exon 21 which increases the likelihood of a response to EGFR-tyrosine kinase inhibitors (EGFR-TKI).

NOTES: Please see page 2 for technical details of the analysis.
REFS: NHS England National Cancer Drugs Fund list. https://www.england.nhs.uk/wp-content/uploads/2017/04/National-Cancer-Drugs-Fund-List-ver1-239.pdf

PREPARED: Clinical Scientist

AUTHORISED: Clinical Scientist

= Patient eligible for EGFR-inhibitor drugs

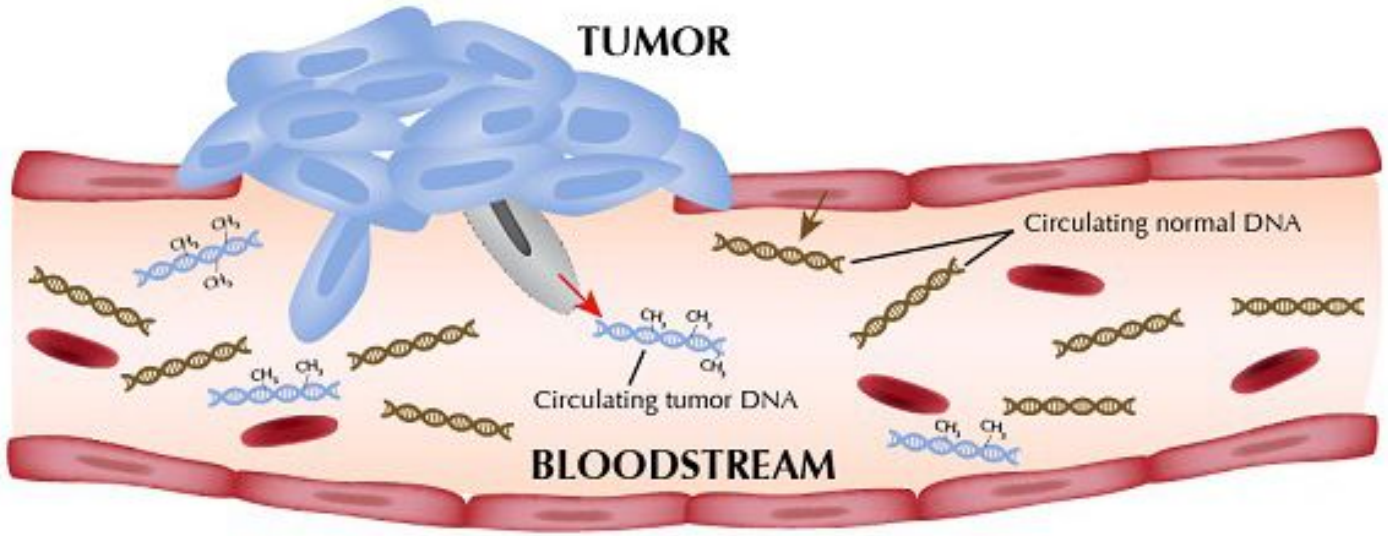
Median overall survival:

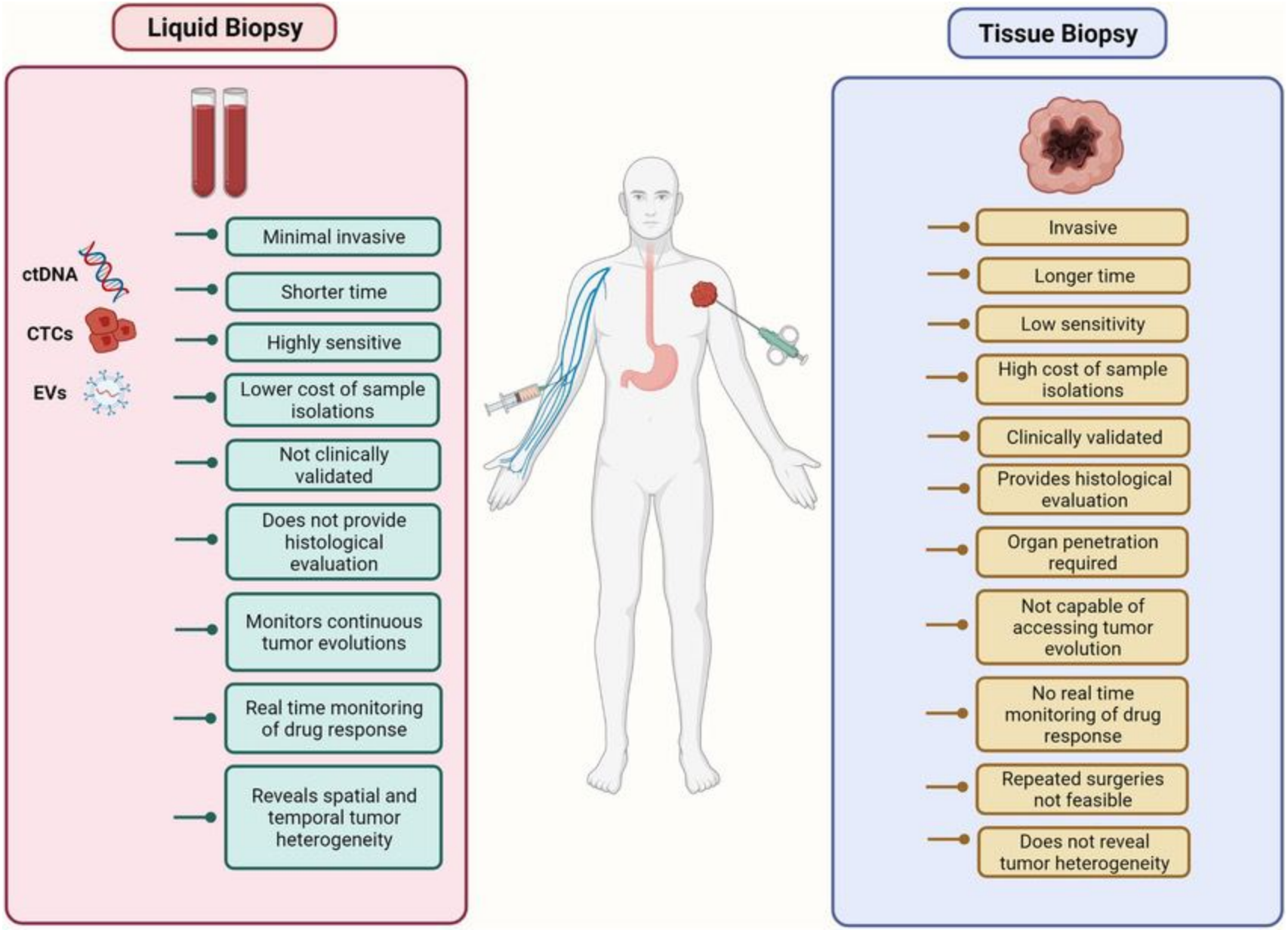
EGFR-mutant: 3-4 years

EGFR-wildtype: ~ 10 months

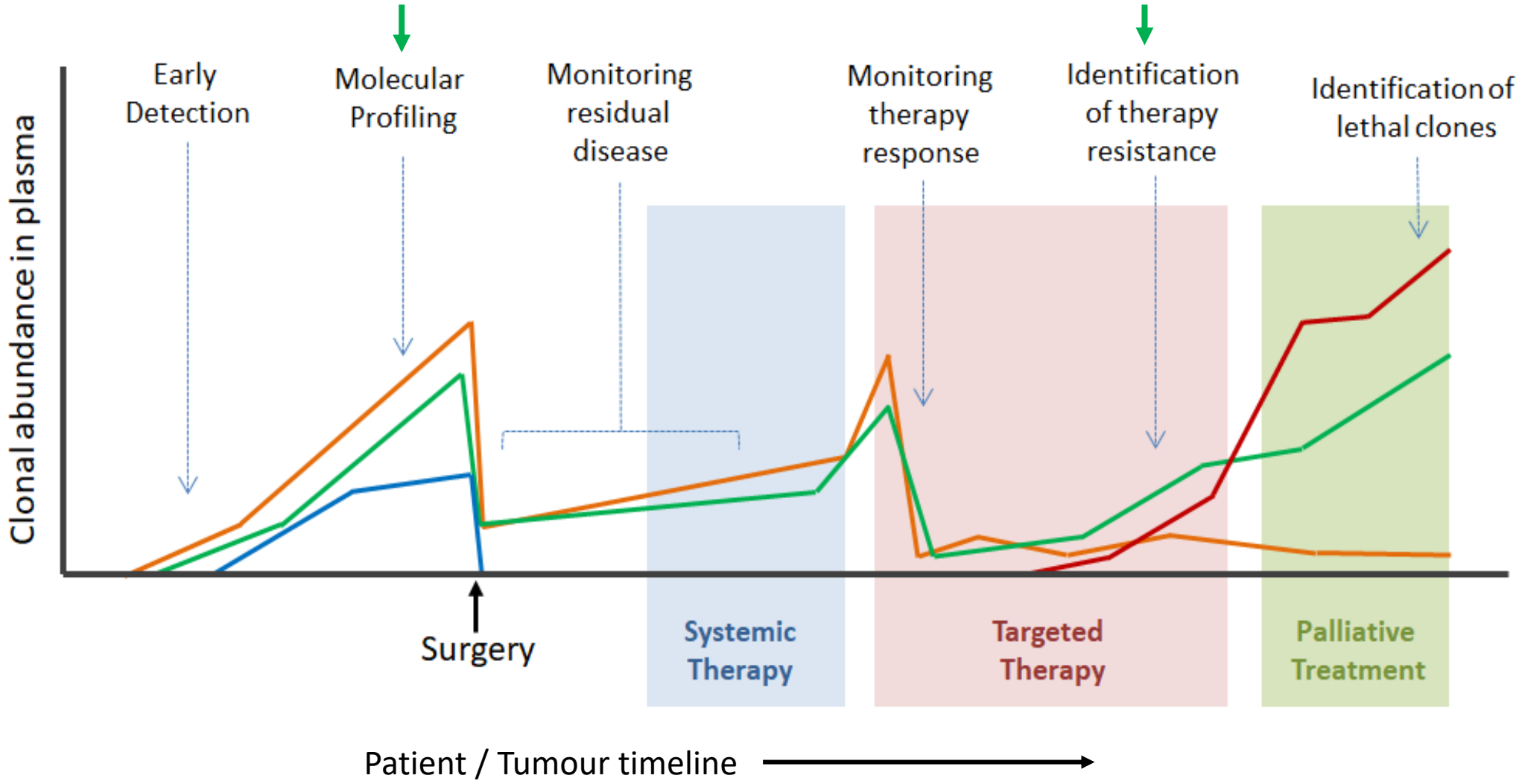
Recent Advances in Genomic Testing…
Circulating tumour DNA

- Cell free DNA (cfDNA) is present in plasma fraction of blood
- Patient's with tumours have more cfDNA in plasma
- Proportion of cfDNA comes from the tumour ctDNA
- Known as 'liquid biopsies'

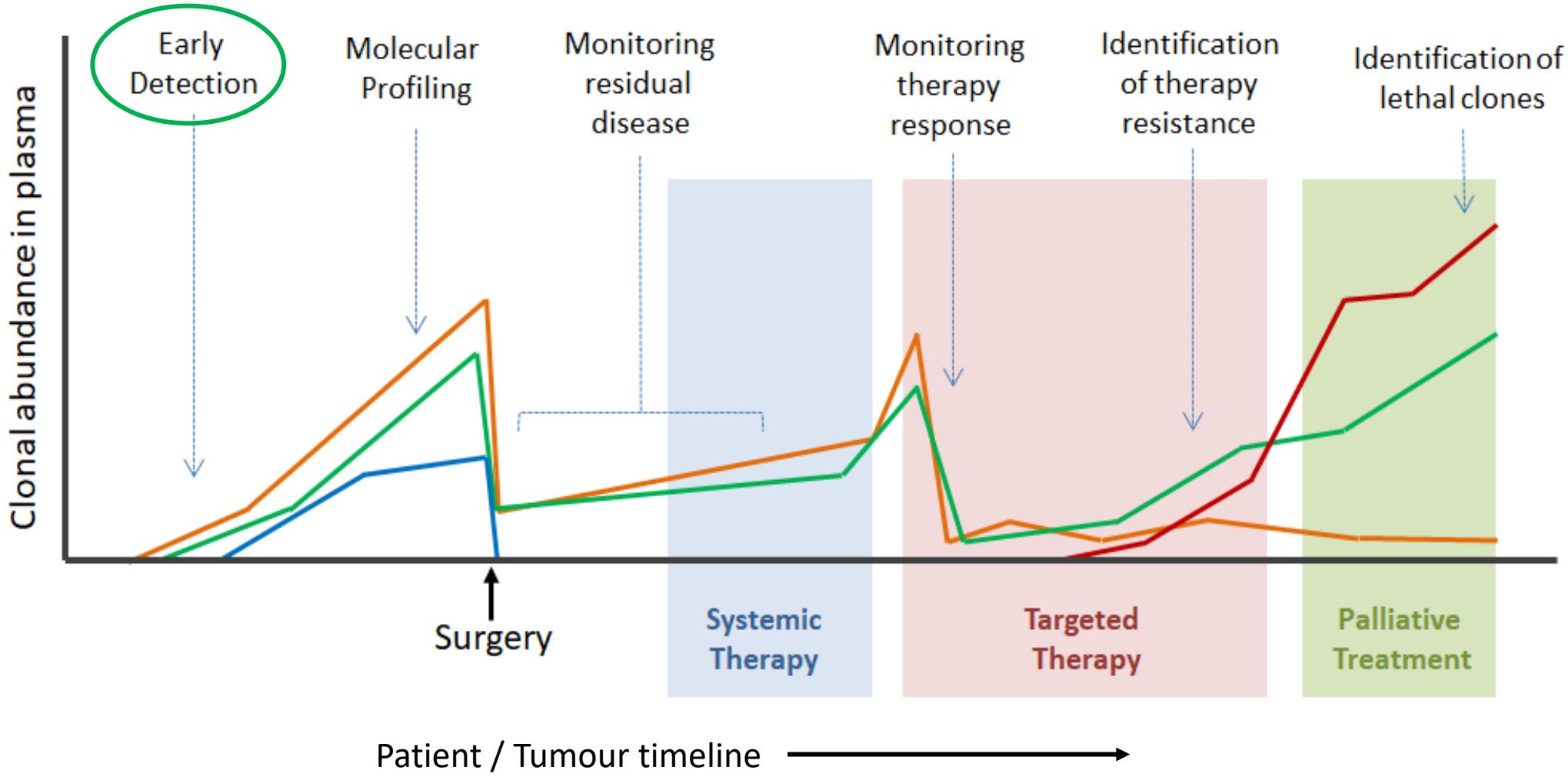



Liquid Biopsy
ctDNA
CTCs
EVs
Minimal invasive
Shorter time
Highly sensitive
Lower cost of sample isolations
Not clinically validated
Does not provide histological evaluation
Monitors continuous tumor evolutions
Real time monitoring of drug response
Reveals spatial and temporal tumor heterogeneity
Tissue Biopsy
Invasive
Longer time
Low sensitivity
High cost of sample isolations
Clinically validated
Provides histological evaluation
Organ penetration required
Not capable of accessing tumor evolution
No real time monitoring of drug response
Repeated surgeries not feasible
Does not reveal tumor heterogeneity

Uses of Liquid Biopsies

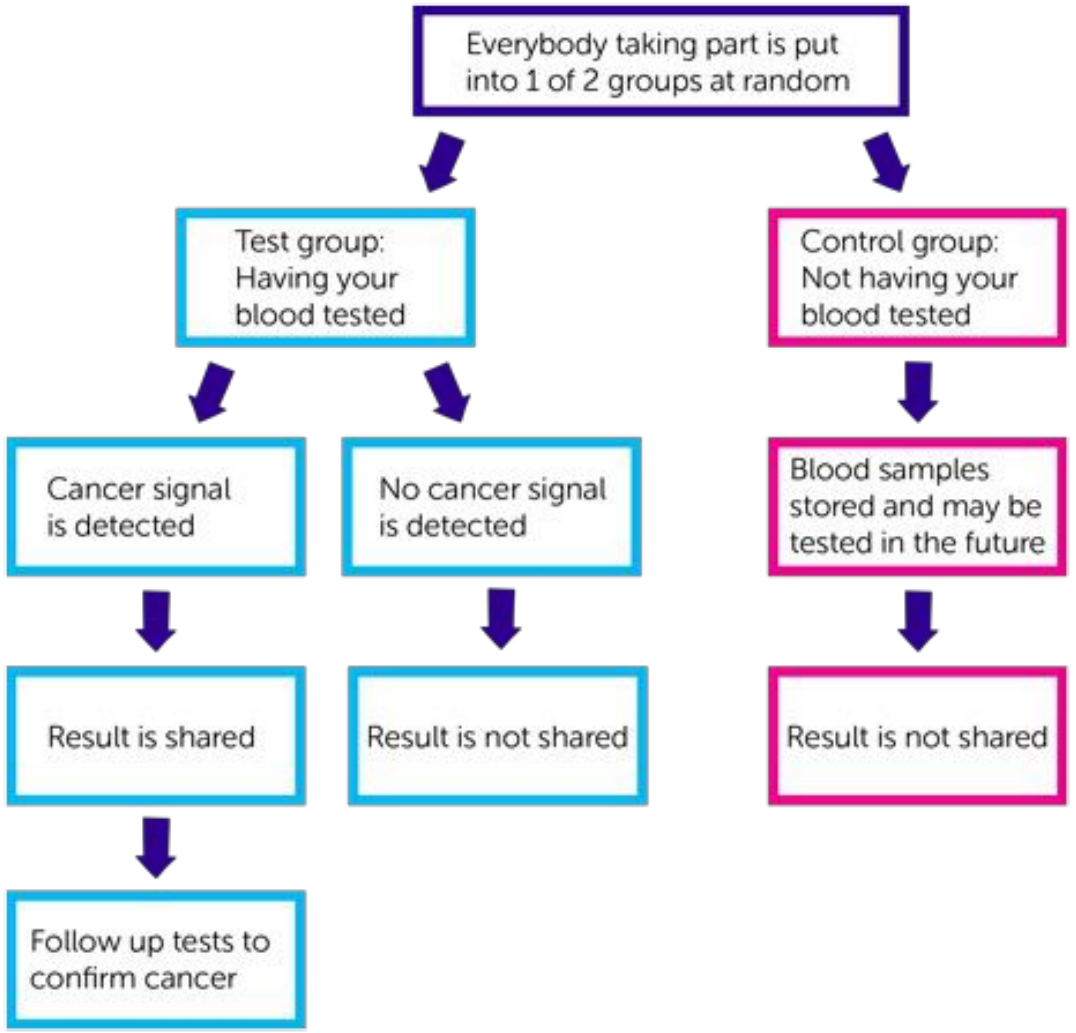


Future Uses of Liquid Biopsies



Galleri Trial

- An **early cancer detection** trial
- Healthy volunteers, aged 50 to 77
- **Galleri®** is a blood test that can detect early signs of many different cancer types
- Shown to detect more than 50 cancer types
- **Positive result** -> further testing to confirm / rule out cancer diagnosis



Thank you!

Any questions?