‘ Molecular ‘ Histopathology
In Era of Individualised Medicine

Dr. Ibrahim Nawroz

Consultant Pathologist

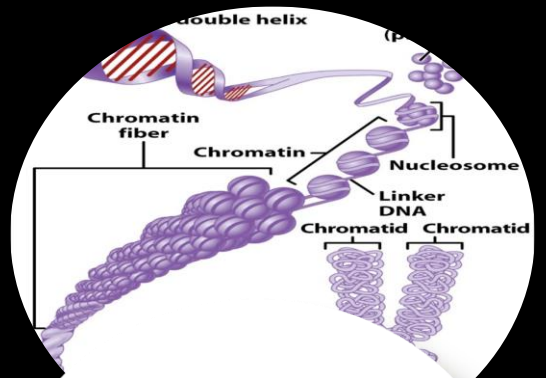
Fife Area Lab.

VHK

HONORARY SENIOR LECTURER ST. ANDREWS MEDICAL SCHOOL

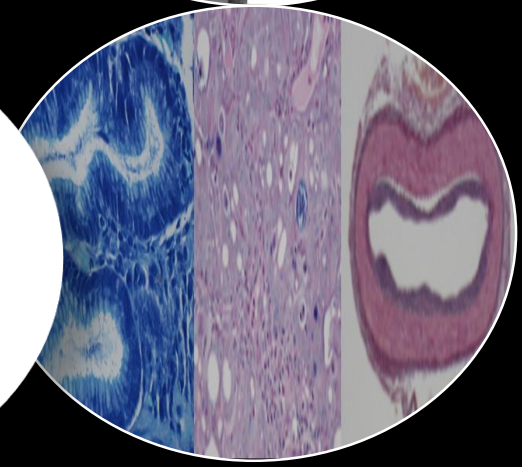
Pathology is the science of study of disease process and is the back bone of modern medicine

would it survive the genomic Era?



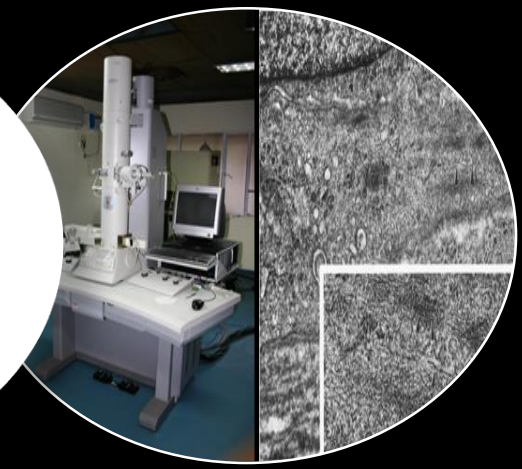
Over last 2 decades

Modern pathology started with the introduction of the compound light microscope in medical practice in 17th century

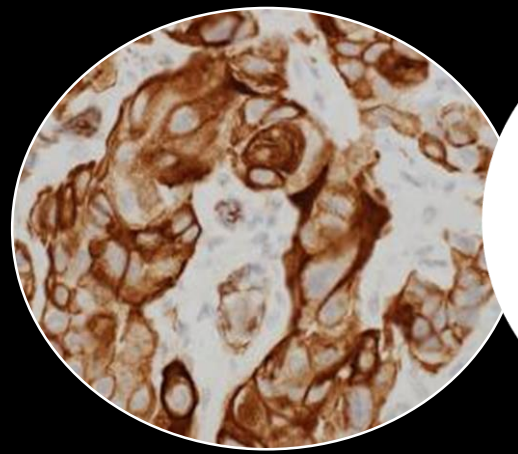


Subsequently the techniques of histochemistry

The techniques of electron /scanning electron microscopy in early 19th century

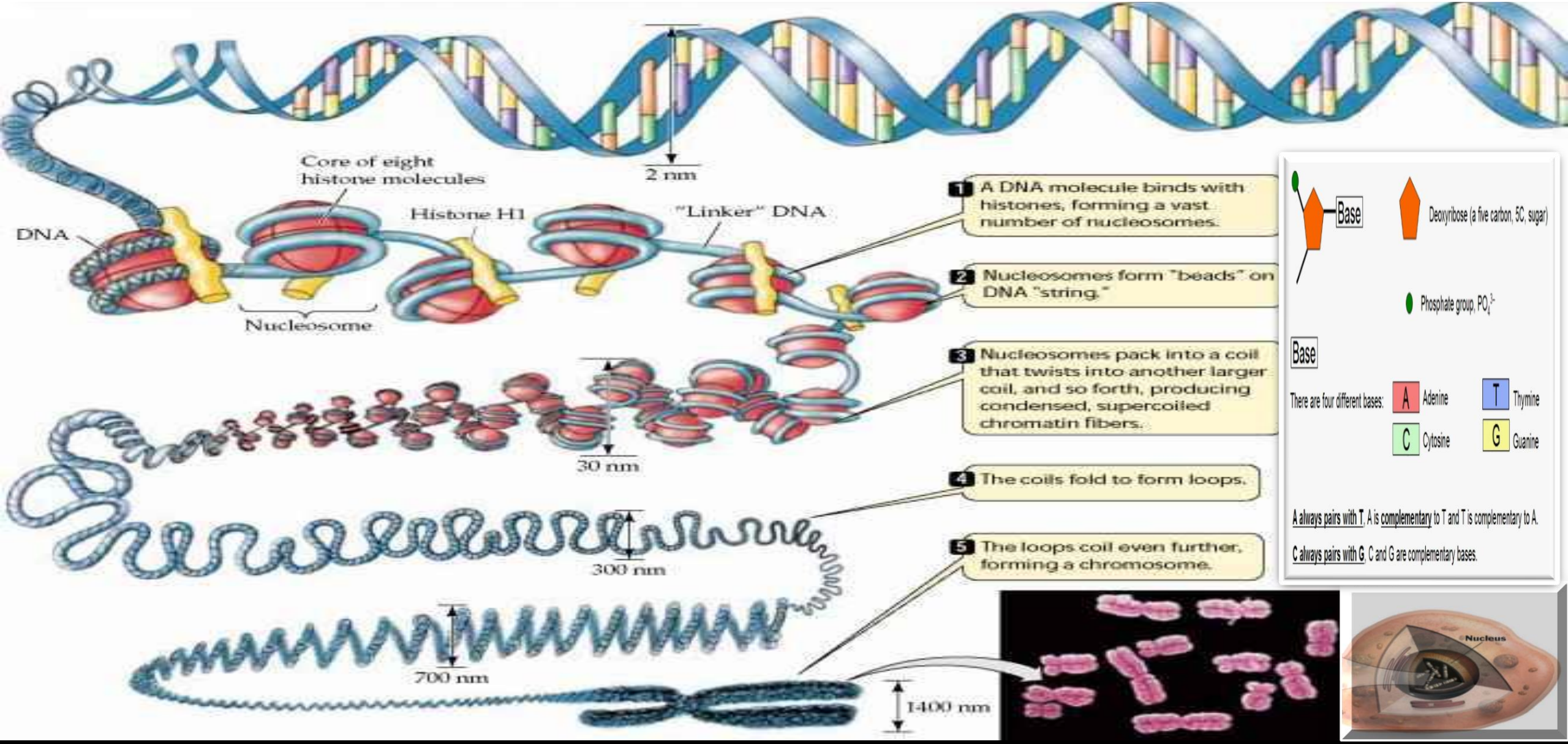


immunohistochemistry, monoclonal antibody production, antigen retrieval, and image analysis, In later ½ 20th century



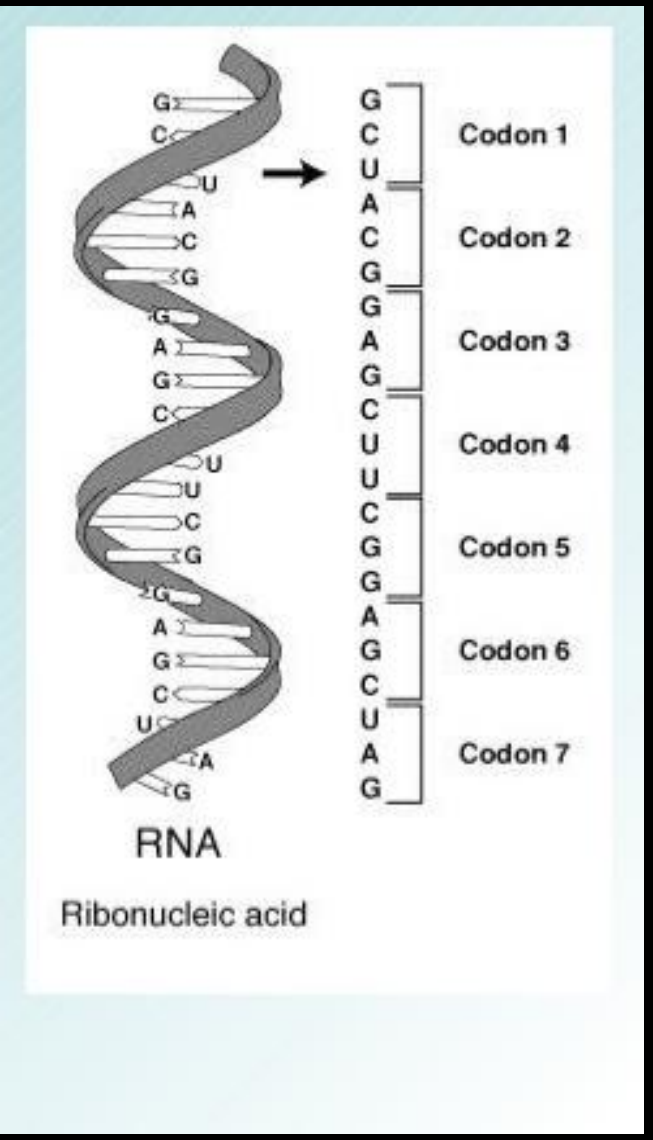
DNA is a **double-helix** of two polymers (strands) of **nucleotides** joined together by **hydrogen bonds** . The strands spiral around one another (double helix).

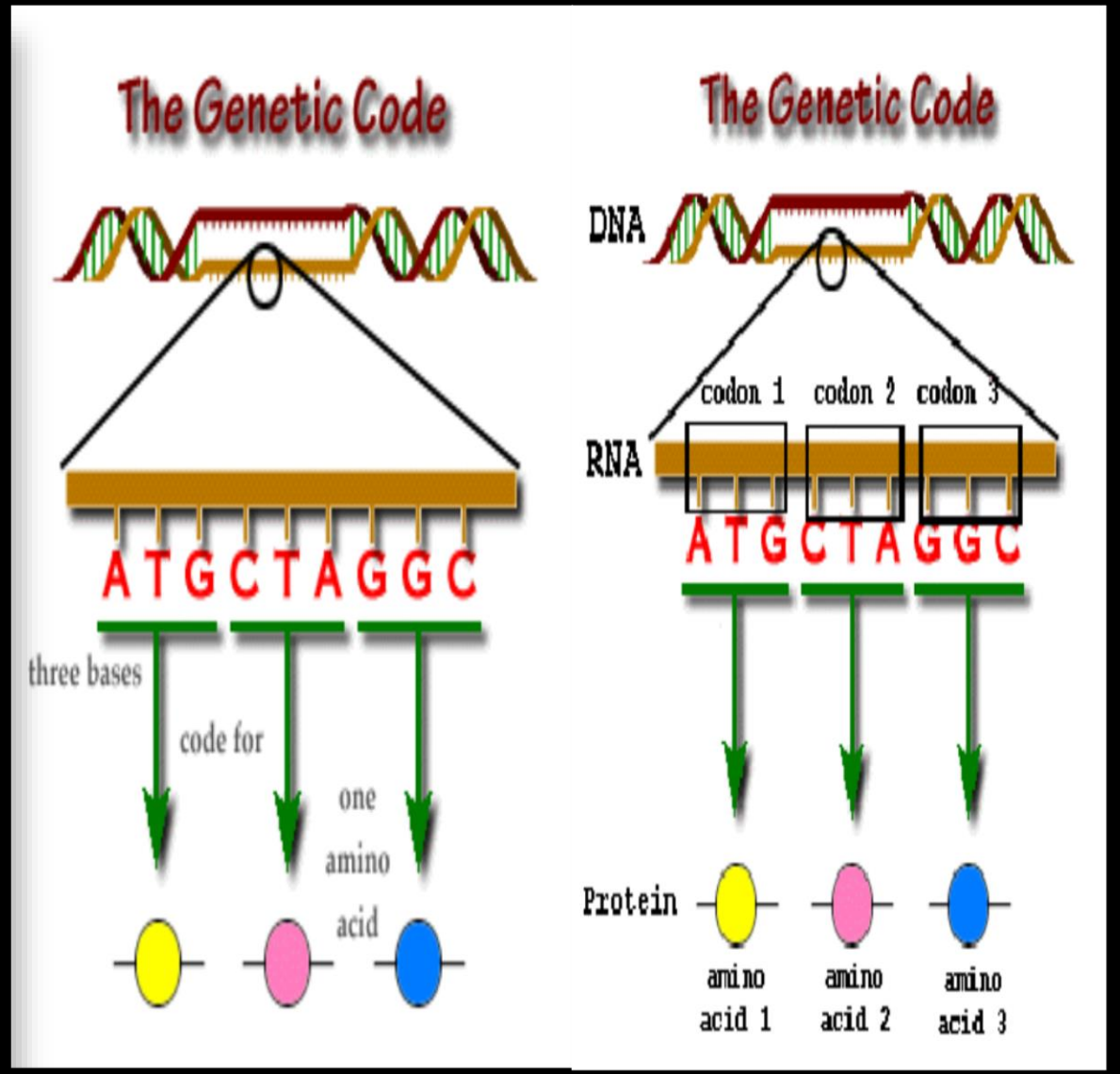
Self- reproducing molecule that caries the instructions to reproduce things fro generation to generation.

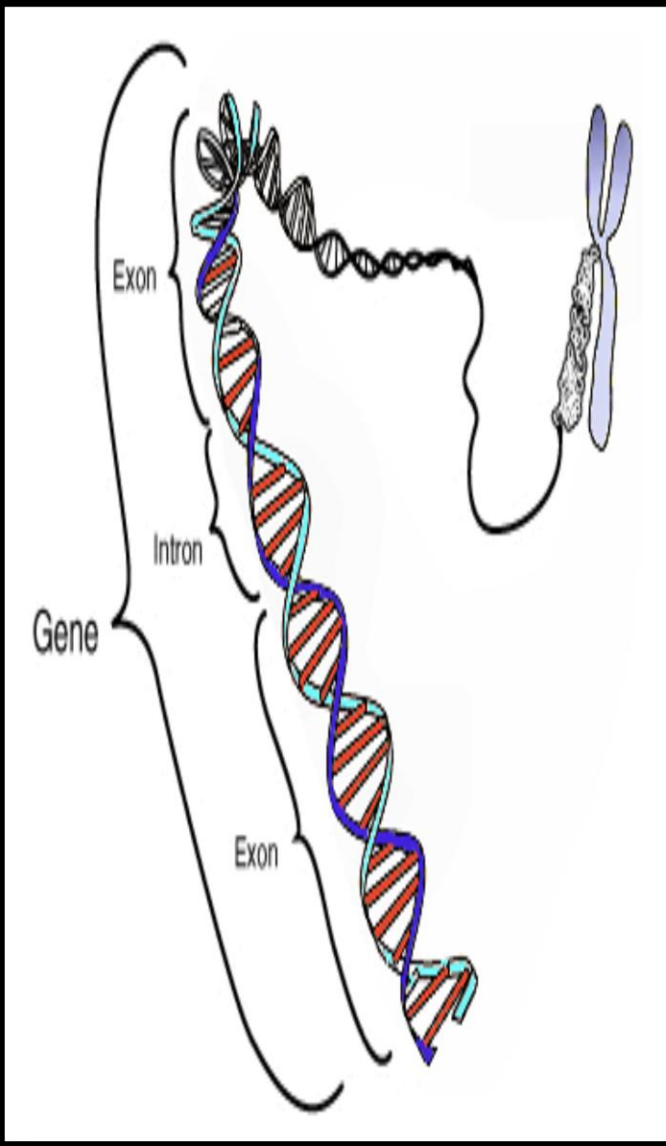


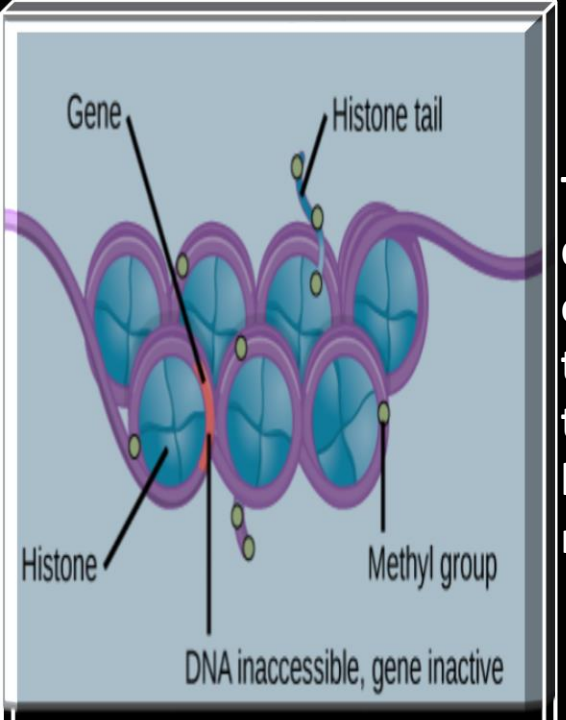
Genetic Code - Set of rules through which the information encoded in the genetic material in living cell is translated into protein

The information encoded in the DNA is in the form of triplet codons, first will be transcribed into RNA then proteins in such way each DNA triplet codon specify one amino acid in the protein

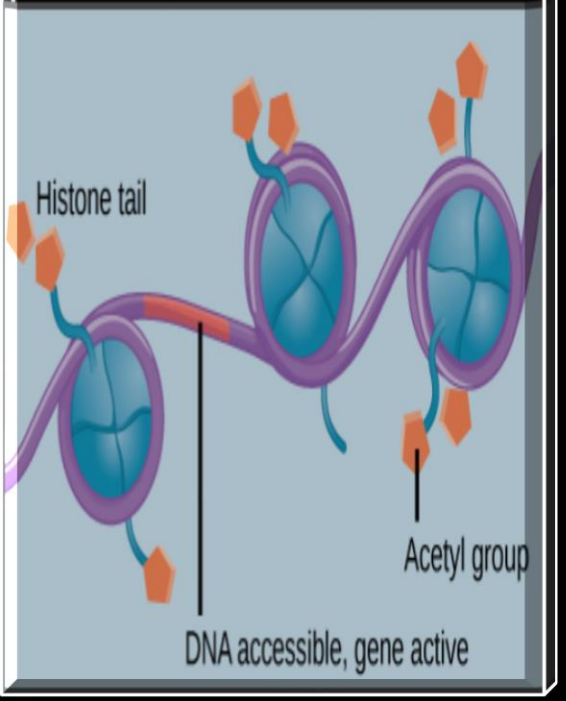




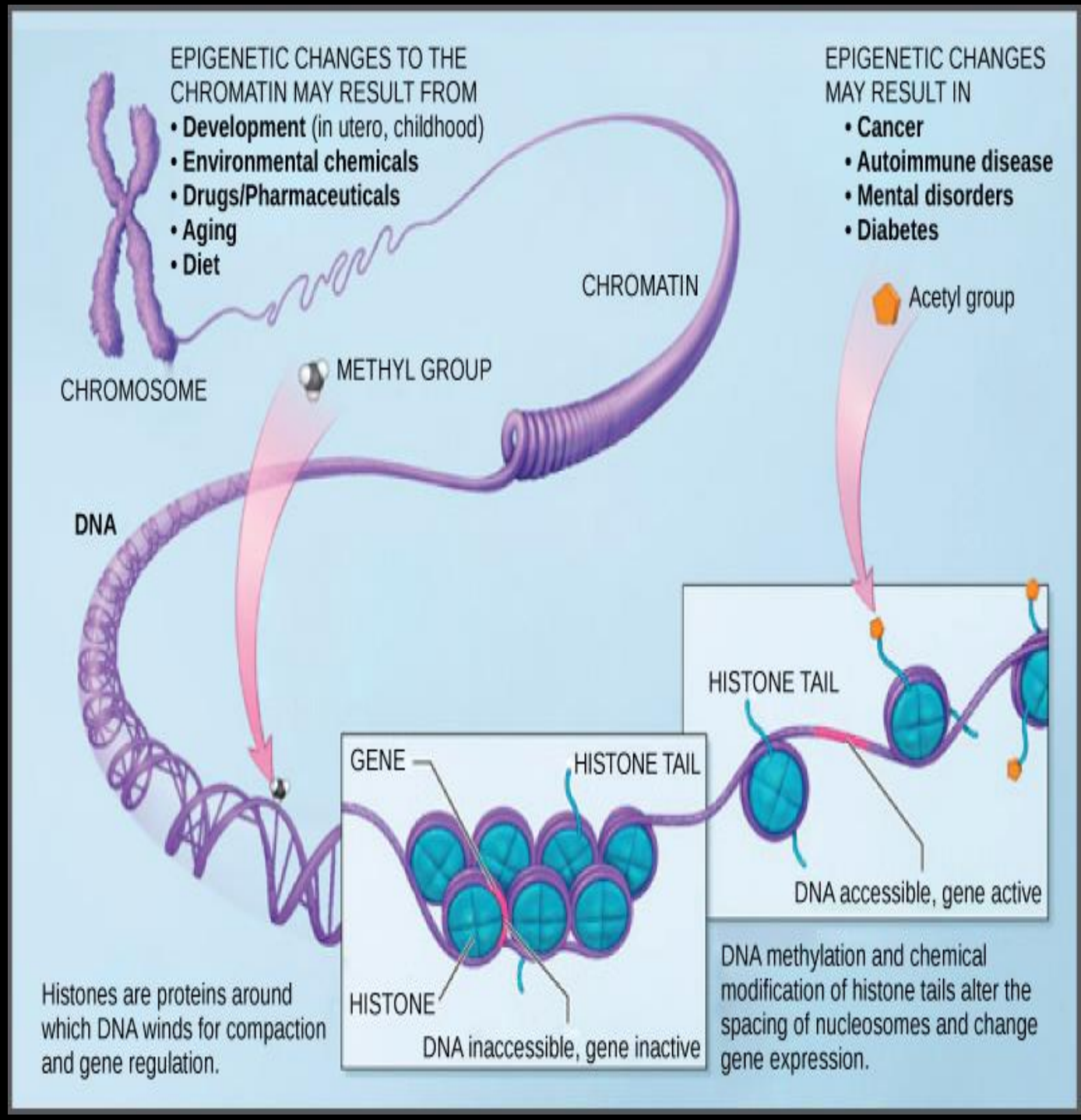




The methyl group and deacetylated histones of DNA causes nucleosomes to pack tightly together as such transcription factors can not bind to DNA and genes will not be exposed.

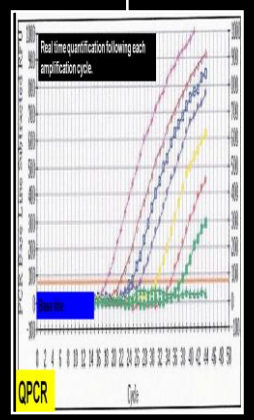


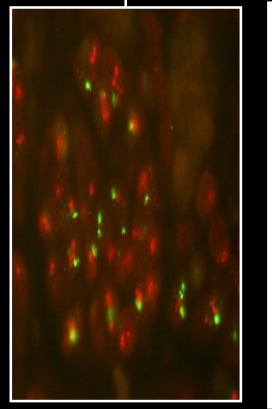
Histone acetylation results in loose packing of nucleosomes as such transcription factors can bind the DNA and genes are exposed

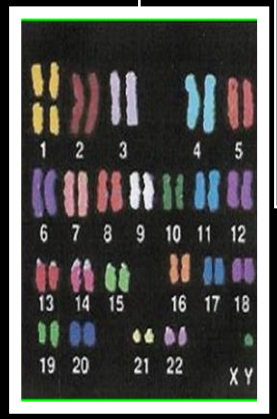


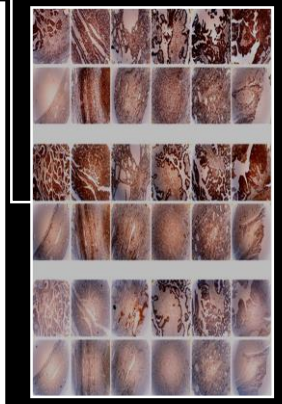
DIAGNOSTIC MOLECULAR PATHOLOGY (DMP)

PCR | FISH,CISH | SKI | TISSUE/GENE MICROARRAY | PROTEOMICS









DNA

Next Generation Sequencing (NGS)

&

Whole Genome Analysis

WGA

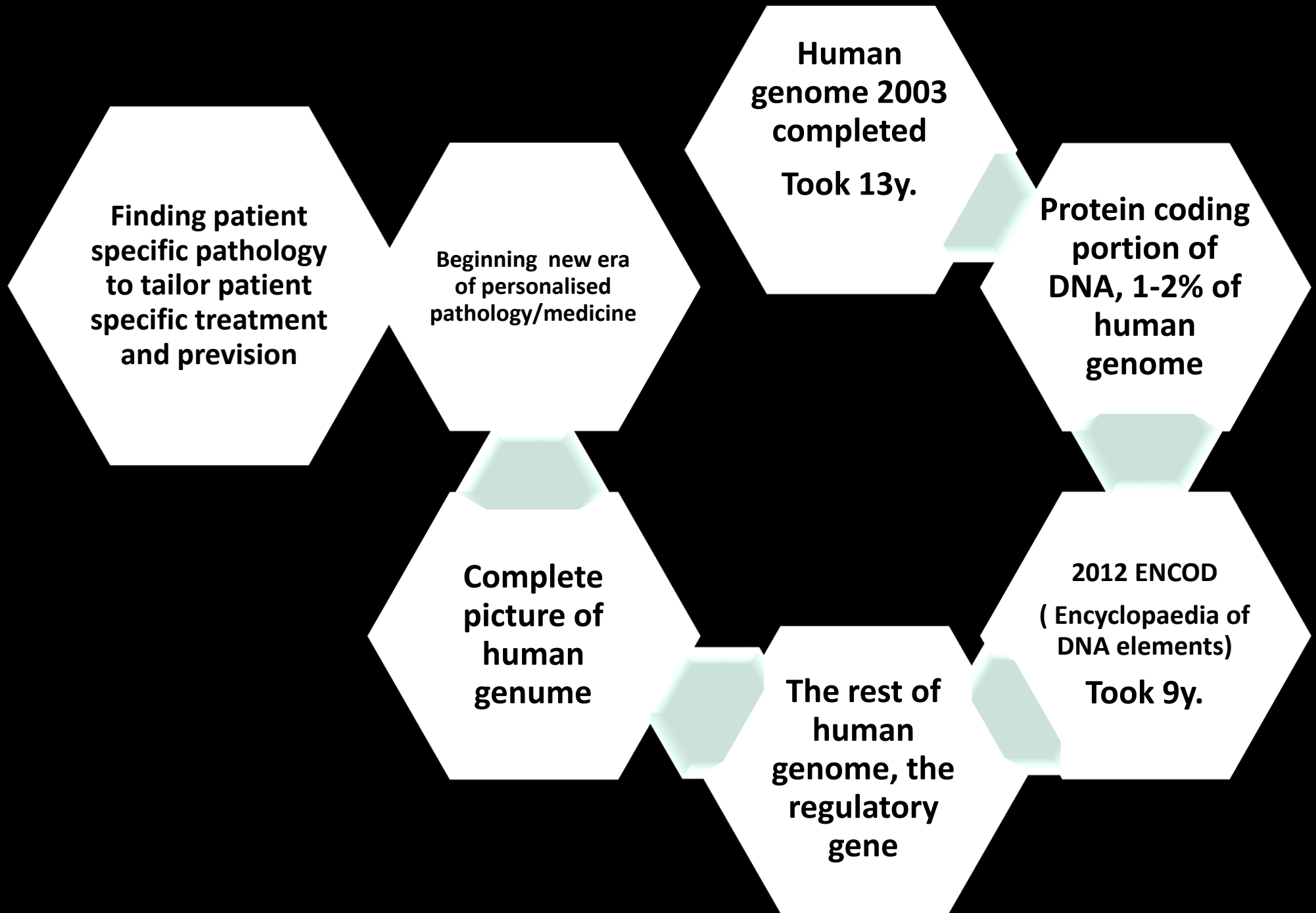
Digital PCR

Comparative genomic hibridisatlion

Mass spectrometry

Microarry technologies etc

DNA /protein high through put technology


Human genome 2003 completed
Took 13y.
Protein coding portion of DNA, 1-2% of human genome
2012 ENCOD
(Encyclopaedia of DNA elements)
Took 9y.
The rest of human genome, the regulatory gene
Complete picture of human genume
Beginning new era of personalised pathology/medicine
Finding patient specific pathology to tailor patient specific treatment and prevision

Cancer Genome

140genes,tumourigenesis driver

71 of which are tumour suppressor genes

54 of which are oncogenes

Any cancer could harbour any thing from 2 up to 12 driver genes mutated, most have 2 - 6 significant mutant genes

Hereditary germline mutations

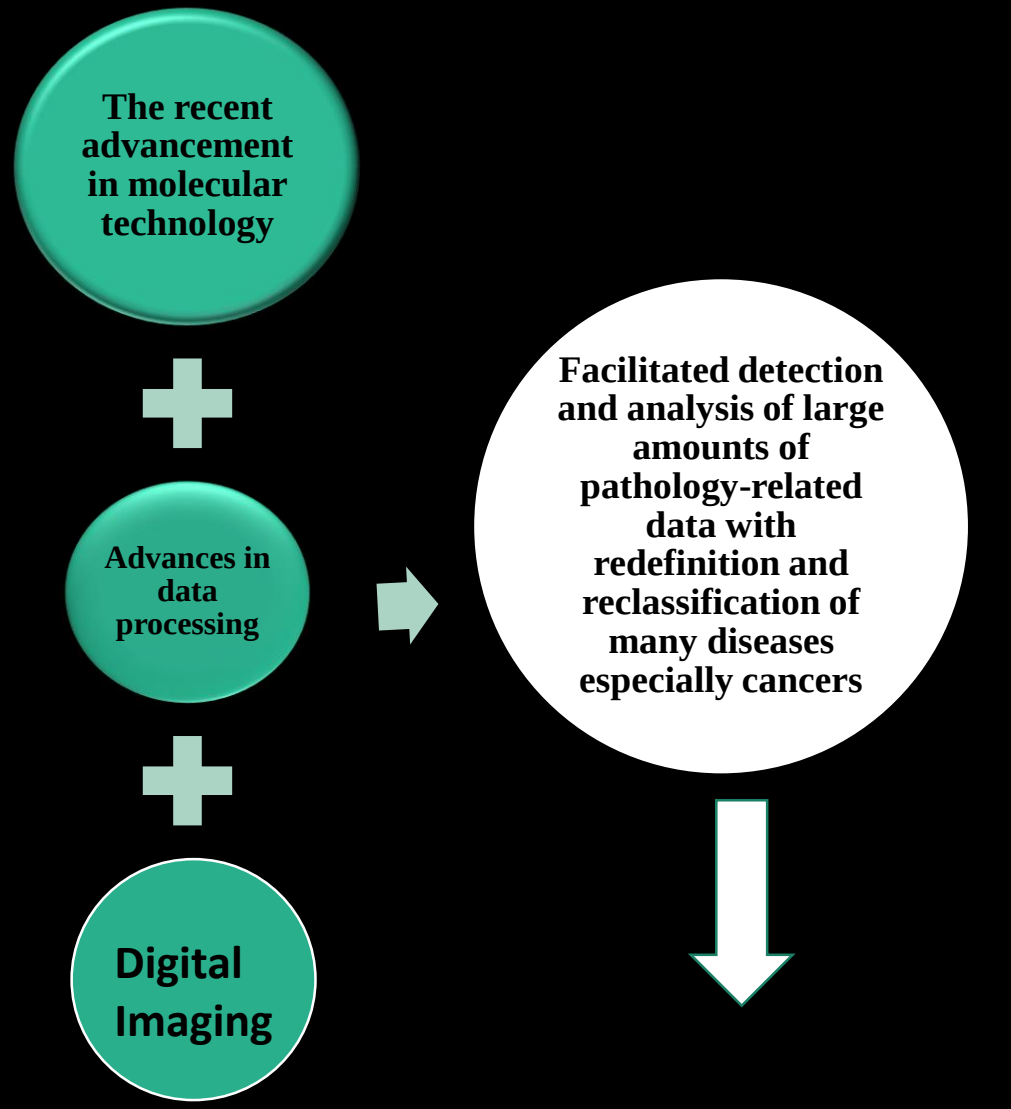
Somatic (acquired) mutations

95% of mutations are single-base substitutions

5% single or few base insertions /deletions

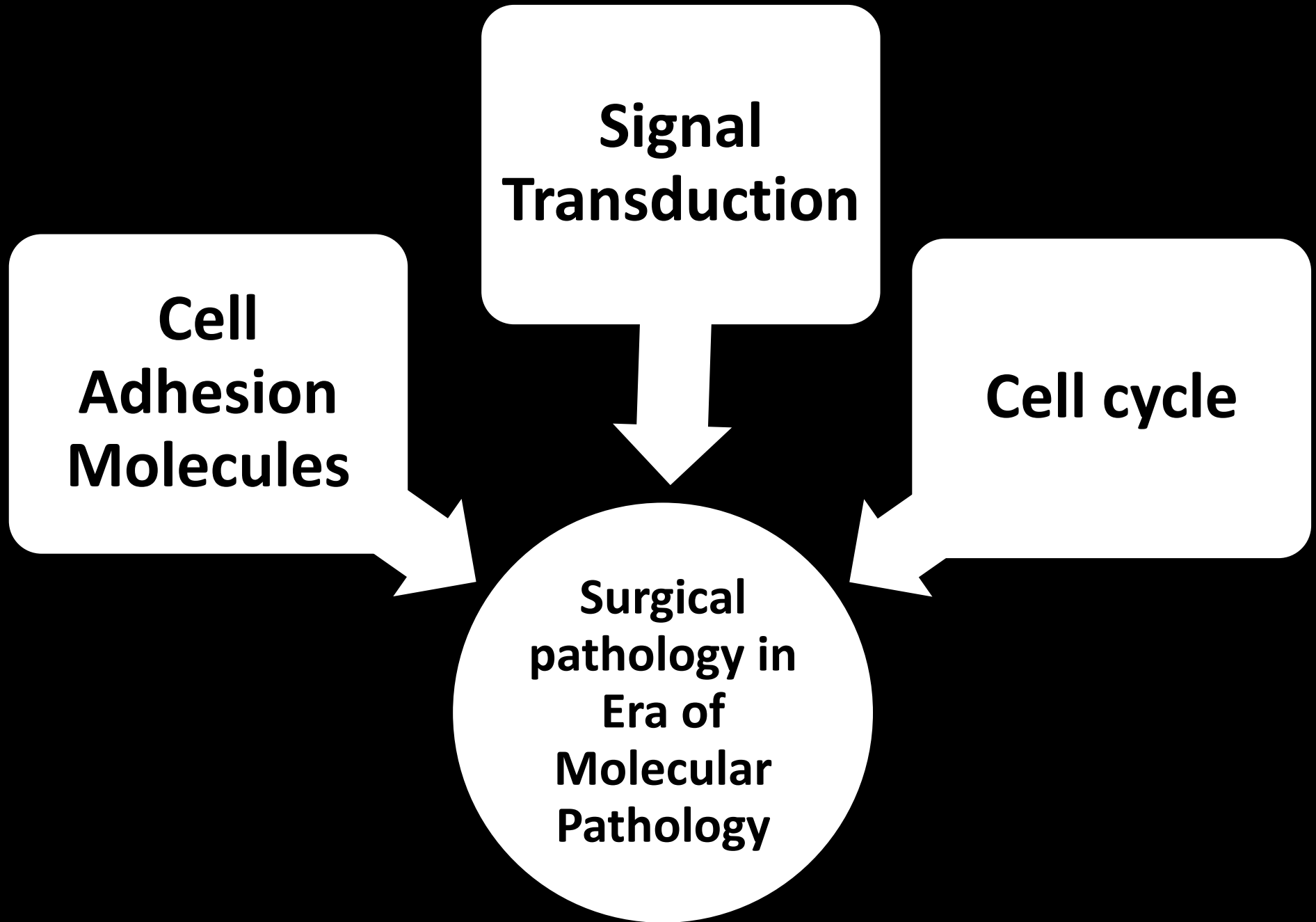
Most mutations are by standers conferring no tumour growth

Few are mutations of driver Genes conferring selective growth effects through intracellular signalling path ways that regulate cell growth, survival and genetic integrity

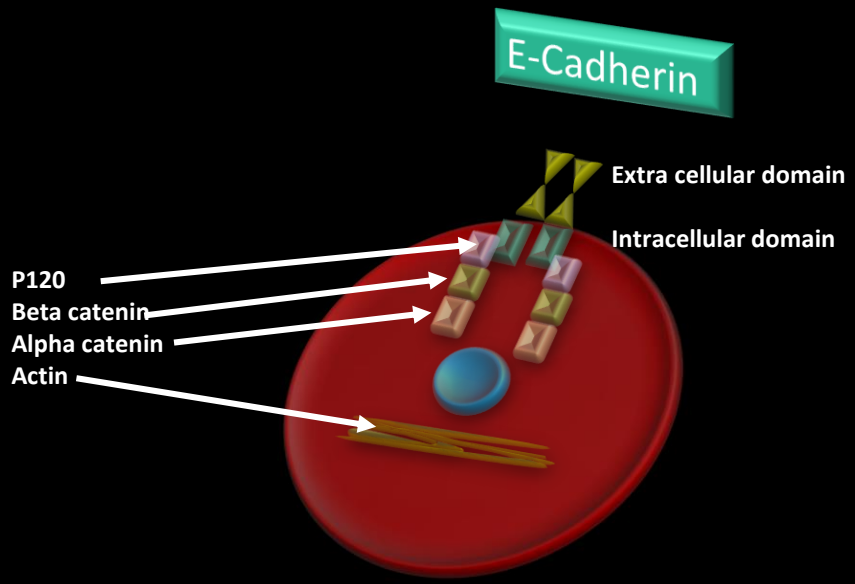


Precision/ Personalized Pathology/Medicine

Molecular Biology



Molecular Biology

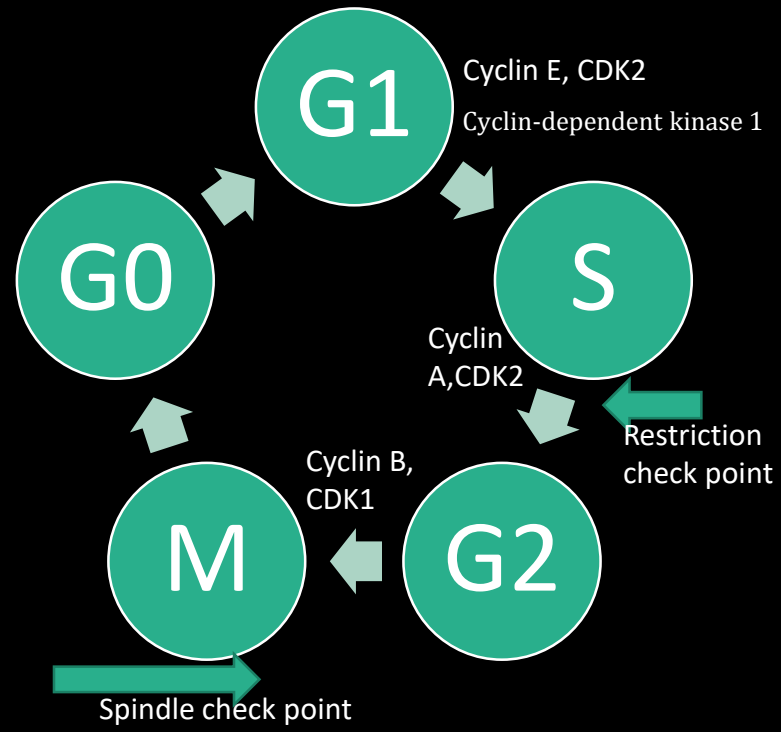


Cell Adhesion Molecules

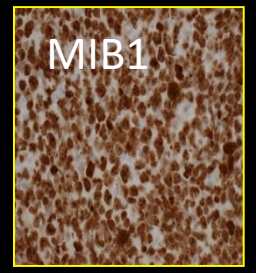
These groups are the :-
1-Classical cadherins
2-The immunoglobulin superfamily
3-integrins
4-selectins.
However, some of these groups are getting very large
and the diversity within groups may lead to further subdivisions.

E-cadherin is one of the best-understood cadherin proteins. In addition to its role in cell adhesion, E-cadherin is involved in transmitting chemical signals within cells, controlling cell maturation and movement, and regulating the activity of certain genes. E-cadherin also acts as a tumour suppressor protein, which means it prevents cells from growing and dividing too rapidly or in an uncontrolled way.

Mutations in the CDH1 gene
which codes for E-cadherin, have been identified
in familial human gastric cancer and lobular breast carcinoma.
E-cadherin can also be silenced by Hypermethylation of the CDH1 Promoter.

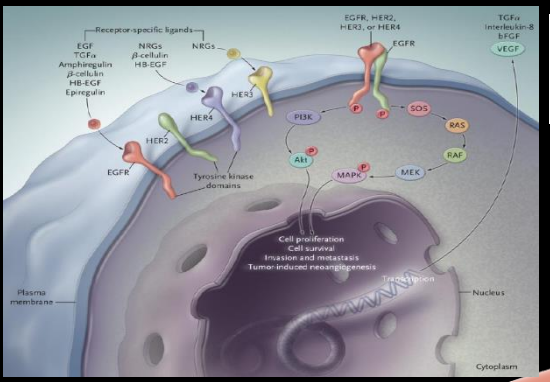


The cell cycle
DNA damage check points causes cell-cycle arrest at the G1/S and G2/M transition and prolonged delay in S phase.



Cell cycle

Signal Transduction



Ciardiello F, Tortora G. *N Engl J Med. 2008;358:1160-1174.* 2008 *Massachusetts Medical Society.*

PDGF, FGF

EGFR

P P

MAPK – Mitogen Activation Protein Kinase

PI3K/PTEN/Akt pathway

MAPK pathway

RAS

ARAF BRAF CRAF

PI3K

PTEN

mTOR

Akt

MEK1/2

Cell growth,prolife ration,survival

ERK1/2

Cycl.D1

RPS6K

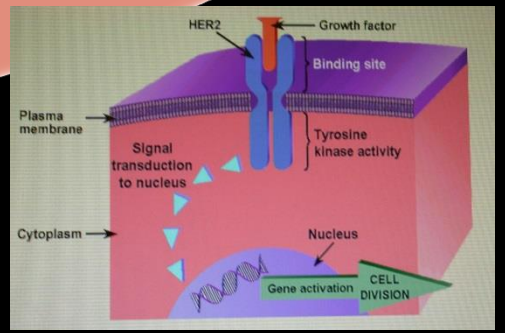
CELL SURVIVAL AND MOTILITY

Cyc Fos Elk1 Ets

Transcription Factors

PI3K – Phosphoinositide 3-Kinase
PTEN – Phosphatase and tensin homolog
Akt – v-akt murine thymoma viral oncogene homolog 1
mTOR - mechanistic Target Of Rapamycin
RPS6K – Ribosomal Protein S 6 Kinase

EGGFR – Epidermal Growth Factor Receptor
PDGFR - Platelet Derived Growth Factor Receptor
FGFR - Fibroblast Derived Growth Factor Receptor



While the DNA genome harbours the information archive, the functional aspect of the cells are controlled through proteins.

Signal transduction controls vital biological functions like growth, death, cellular movement, localisation, differentiation etc.

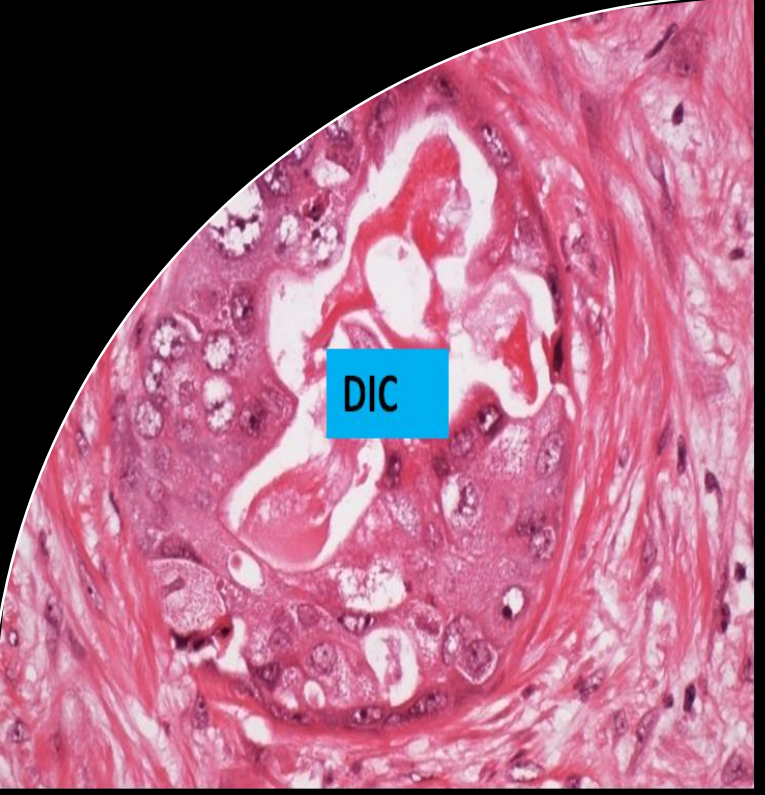
This signal transduction is entirely epigenetic controlled by protein , enzyme activity.

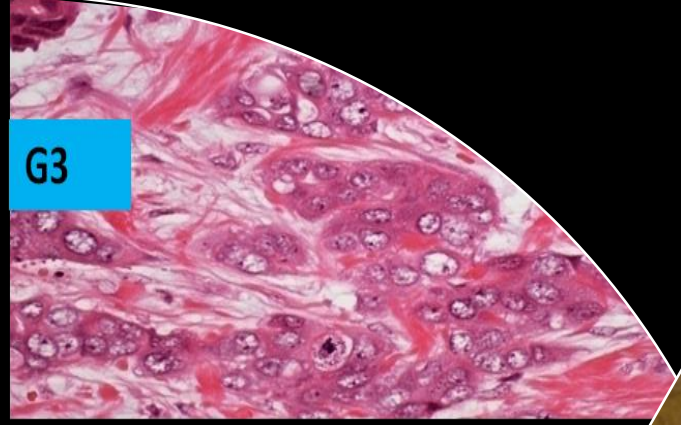
Cancers while based on genetic mutation, functionally manifest as dysfunctional protein signal transduction.

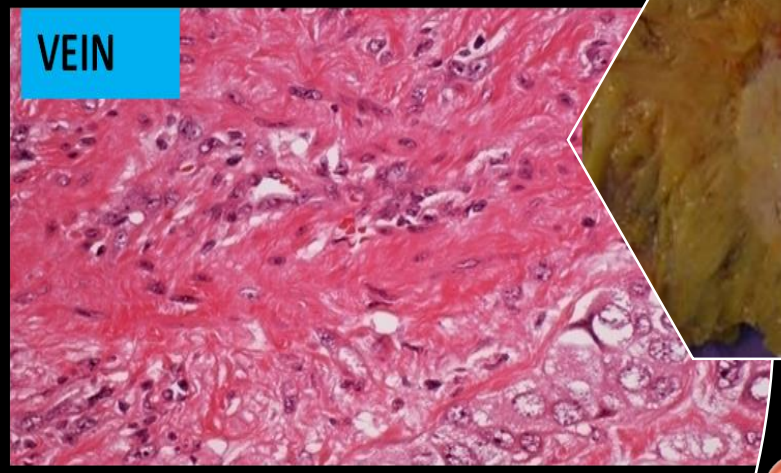
Immunohistochemical, FISH & CIH identification and interpretation are prominent remit of diagnostic surgical pathology,

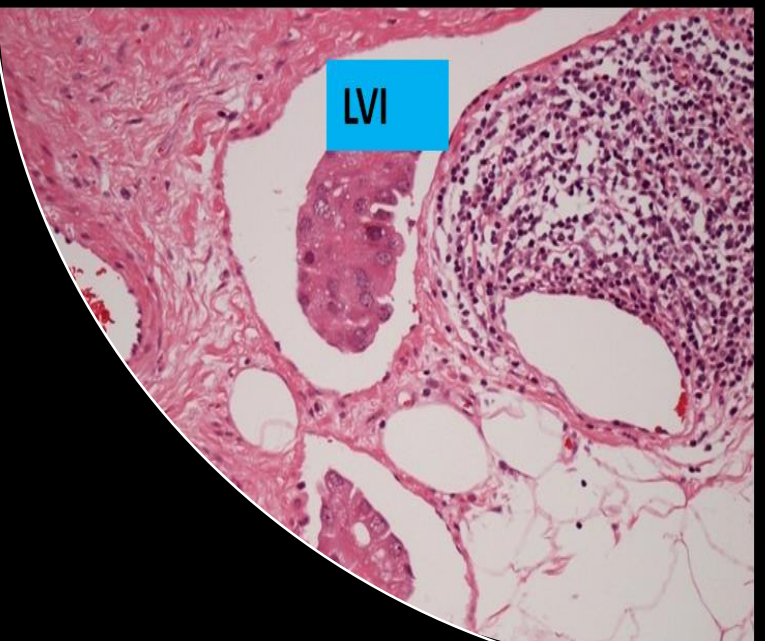
The pharmacological intervention modulates the aberrant protein activity but not the genetic defect.

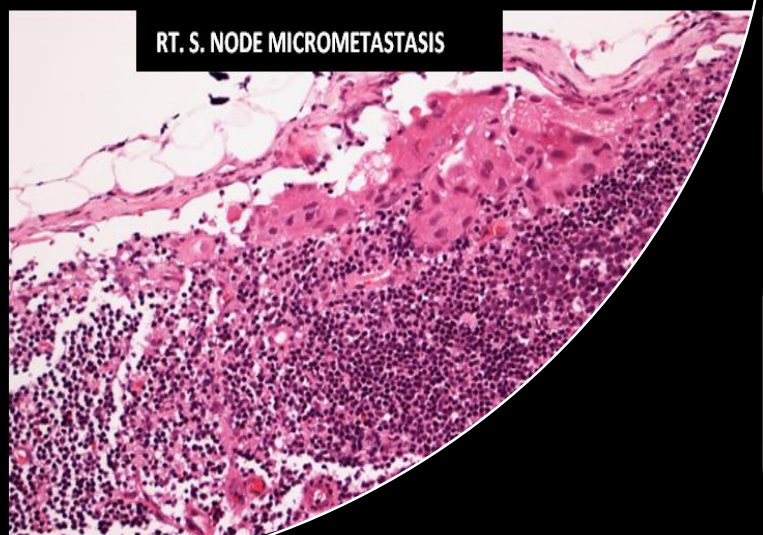
1- Invasive duct carcinoma ,nos 80%
2- Invasive lobular carcinoma 10-15%
3- Invasive duct Ca. Special type
4- Others

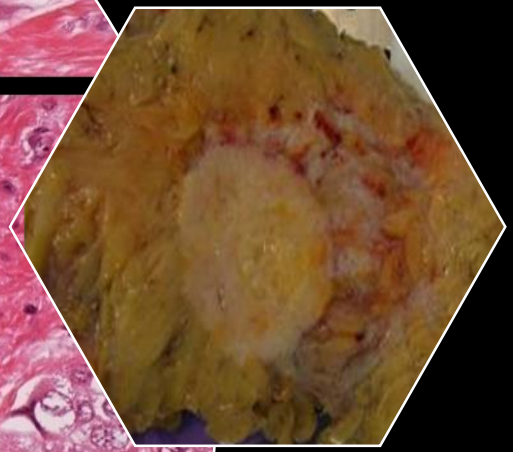


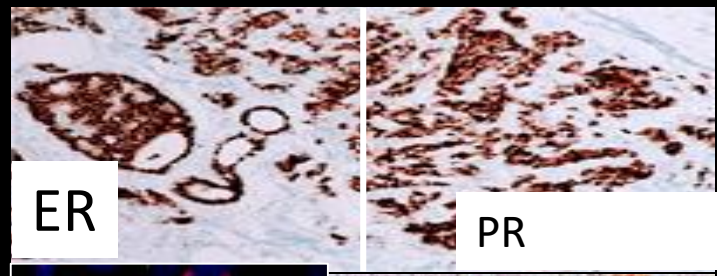


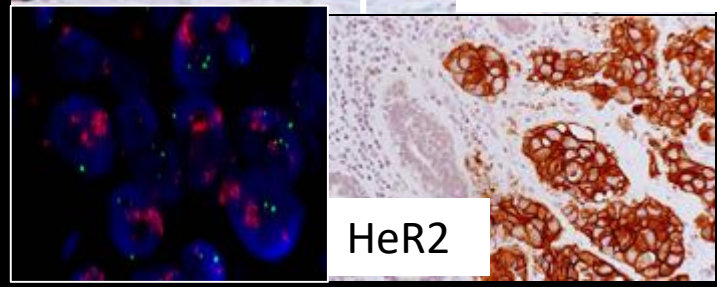












Up 2005

- Recurrence risk was main determinant
- Average for all

FROM 2005

- Emphasis on
- Target

PCR

Oncotype DX Gene profiler

21 gene signature including five reference genes and 16 test genes belonging to ER, HER2, proliferation, invasion and miscellaneous groups identifies three risk groups

Indicated for :-
stage I, II , ER + NODE – VE Carcinoma
DIC

RS <18 → **CANCER OR DIC OF LORW RISK RECURRANCE** → **Carcinoma Benefit from chemotherapy is likely to be small not outweigh risk of side effects** → **DIC benefit from radiotherapy is small not outweigh the risk of side effects**

RS 18-30 → **CANCER OR DIC OF INTERMEDIAT RISK RECURRANCE** → **Carcinoma Benefit from chemotherapy is unclear if it outweighs risk of side effects** → **DIC benefit from radiotherapy is unclear if outweighs the risk of side effects**

RS 31-100 → **CANCER OR DIC OFHIGH RISK RECURRANCE** → **Carcinoma Benefit from chemotherapy likely to be greater than risk of side effects** → **DIC benefit from radiotherapy is likely to be greater than the risk of side effects**

B – Cell Lymphoma Targeted Therapy

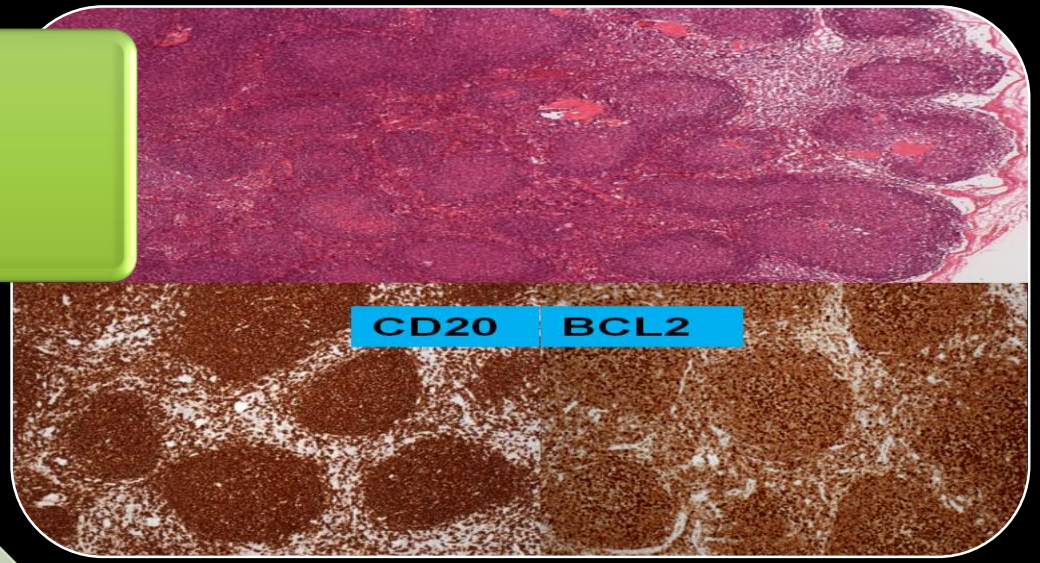
FOLLICULAR LYMPHOMA

CYTOGENETIC

t(14 ; 18)(q21 ; q32)

BCL2 , IGH

> 90%



- **CD20 + B – Cell Lymphoma Targeted Therapy**
- **Anti CD20, Anti CD30 Monoclonal antibodies**
- **Small Molecule Inhibiters (BH3 mimickers) with or without**
- **Standard chemotherapy, anti B-cell monoclonal and mTOR inhibitor rapamycin**

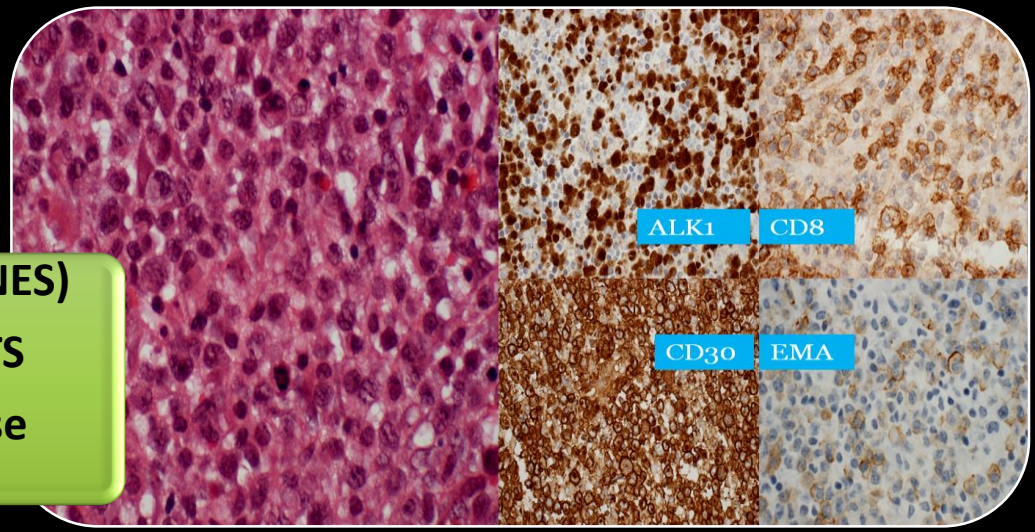
ANAPLASTIC LARGE CELL LYMPHOMA

ALK1

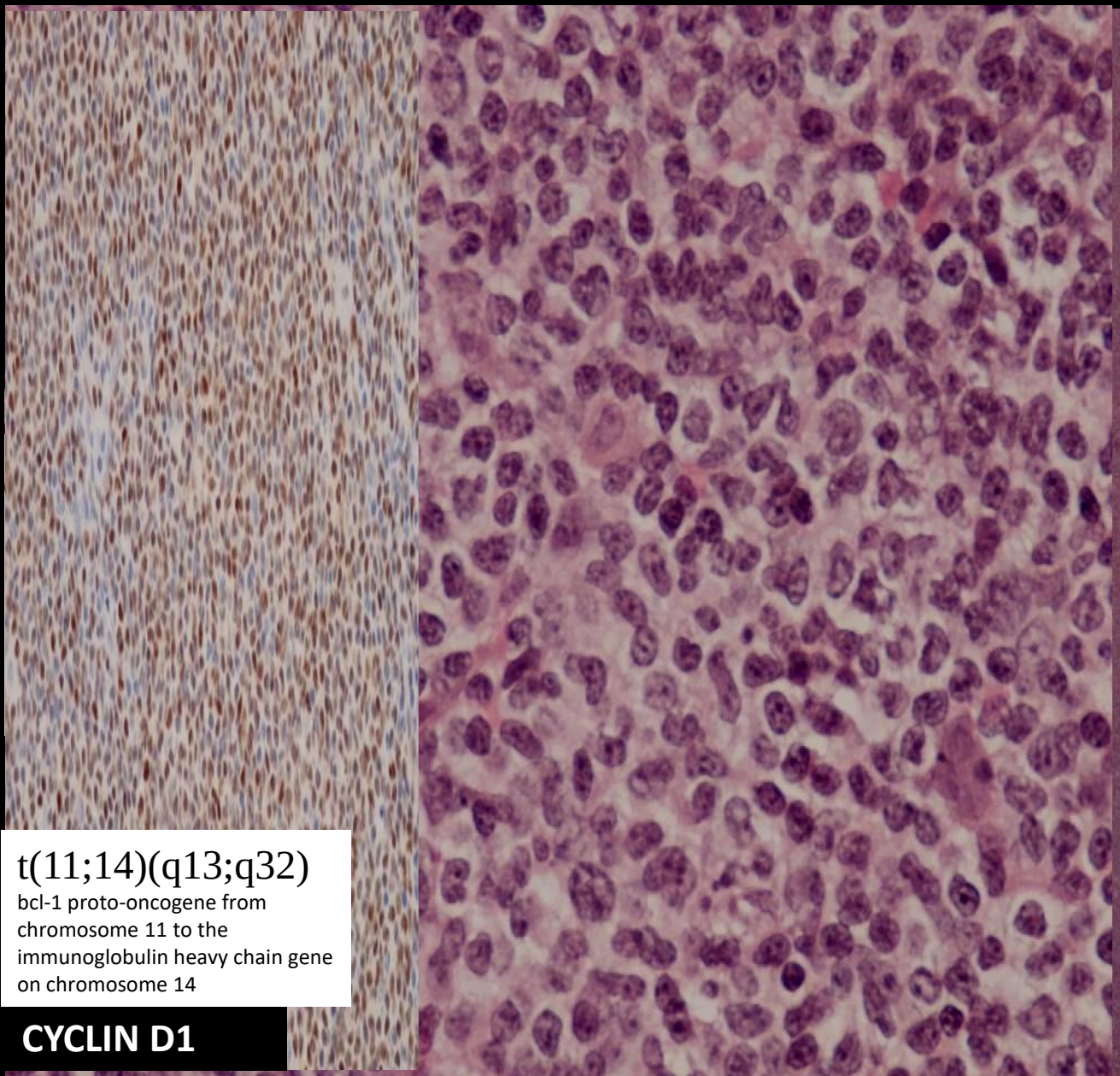
t(2 ; 5)(p23 ; q35) + VARIANTS

NPM , ALK1(GENES) AND VARIANTS

Tyrosine Kinase Inhibitors

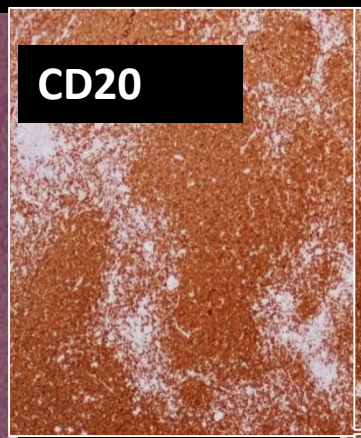


F.67Y.ENLARGED PAINLESS LT. GROIN LYMPH NODE

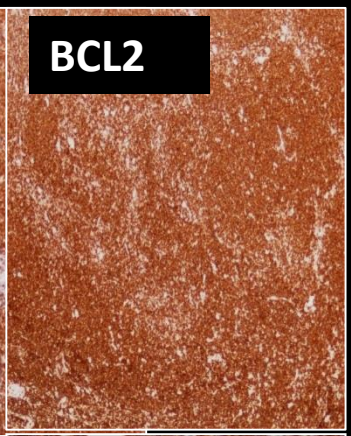


t(11;14)(q13;q32)
bcl-1 proto-oncogene from chromosome 11 to the immunoglobulin heavy chain gene on chromosome 14

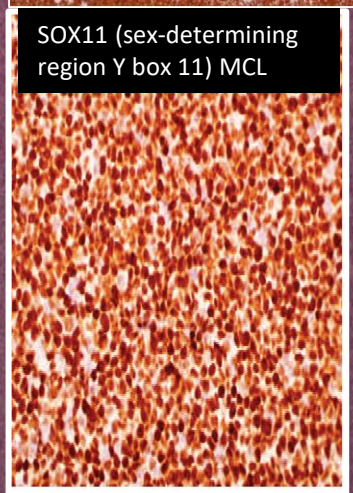
CYCLIN D1



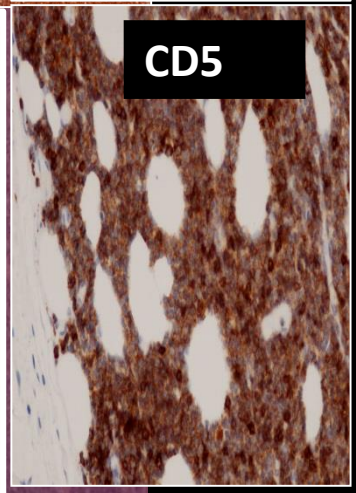
CD20



BCL2



SOX11 (sex-determining region Y box 11) MCL



CD5

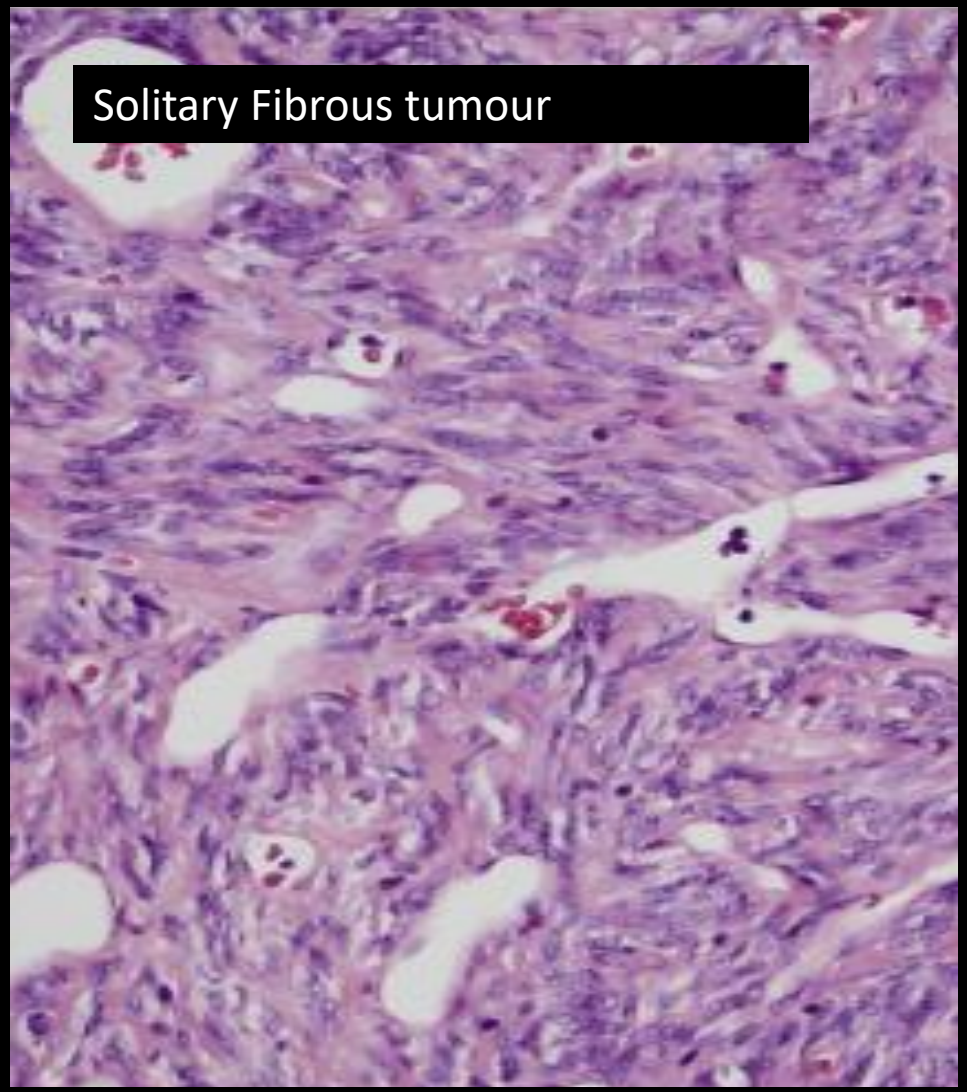
CD20, BL2, CD5, CYCLIN D1 +ve

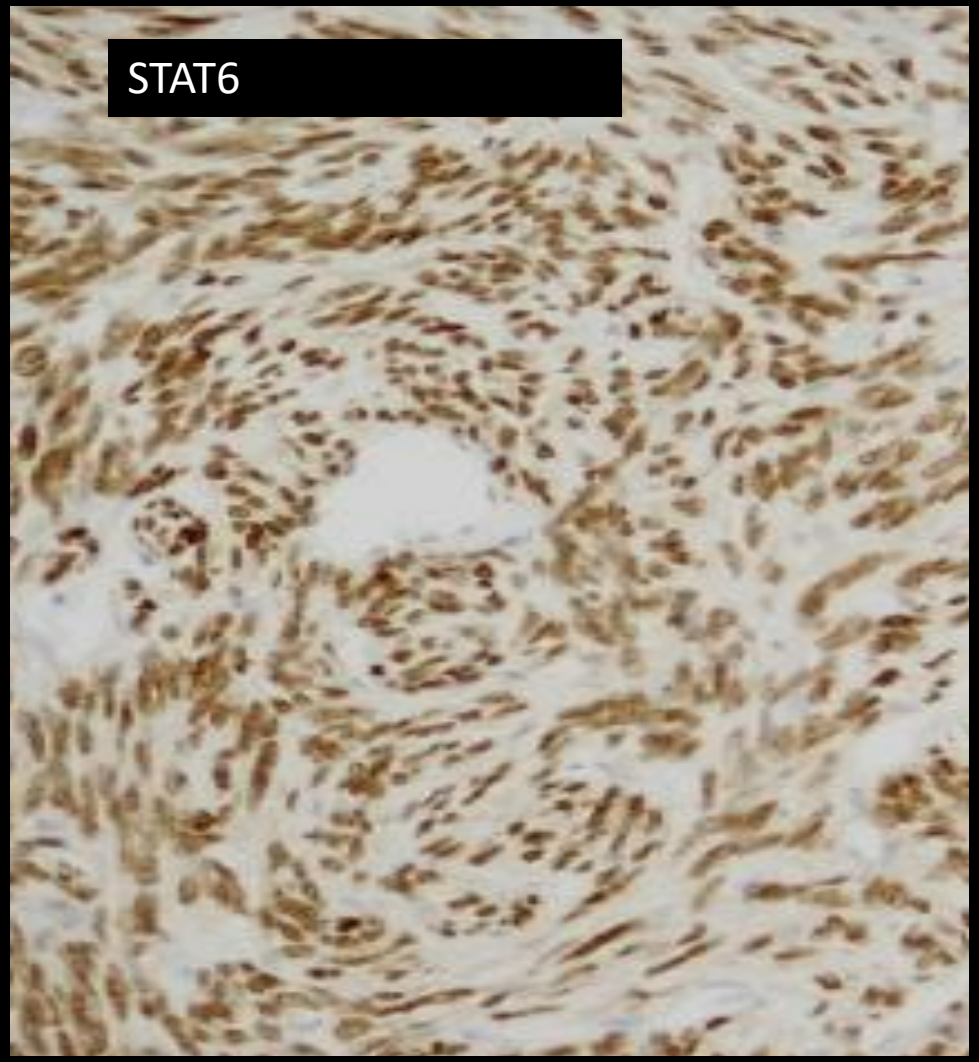
CD10, bcl6, CD138, CD21, CD23 –VE

MONOTYPIC KAPPA +VE

MIB1 PROLiFERATIVE InDEX 20%

B - Mantle Cell Lymphoma





NAB2-STAT6 gene fusion is specific molecular change with EGR1 over expression

Cancer Targeted Therapy

Drugs or substances which interfere with specific molecules involved in cancer cell growth and survival

Directed at cancer specific molecular target, usually cytostatic blocking cancer cell proliferation

Chemotherapy is usually cytotoxic kills rapidly proliferating tumour and normal cells

Potential targets are these that play important role in cancer cell growth and survival

Proteins excessively expressed in cancer cells like HER – 2 Breast cancer.

Mutant protein in cancer cells that drives cancer progression like BRAF (BRAFV600E) protein in Melanoma

Chromosomal abnormality in cancer cell the product of which is fusion protein like BCR-ABL fusion protein in CML

Types of Targeted therapy

Hormonal therapies

Signal transduction inhibitors

-Small Molecule Inhibiters

Monoclonal antibodies (humanised)

Gene expression modulators

Apoptosis inhibitors

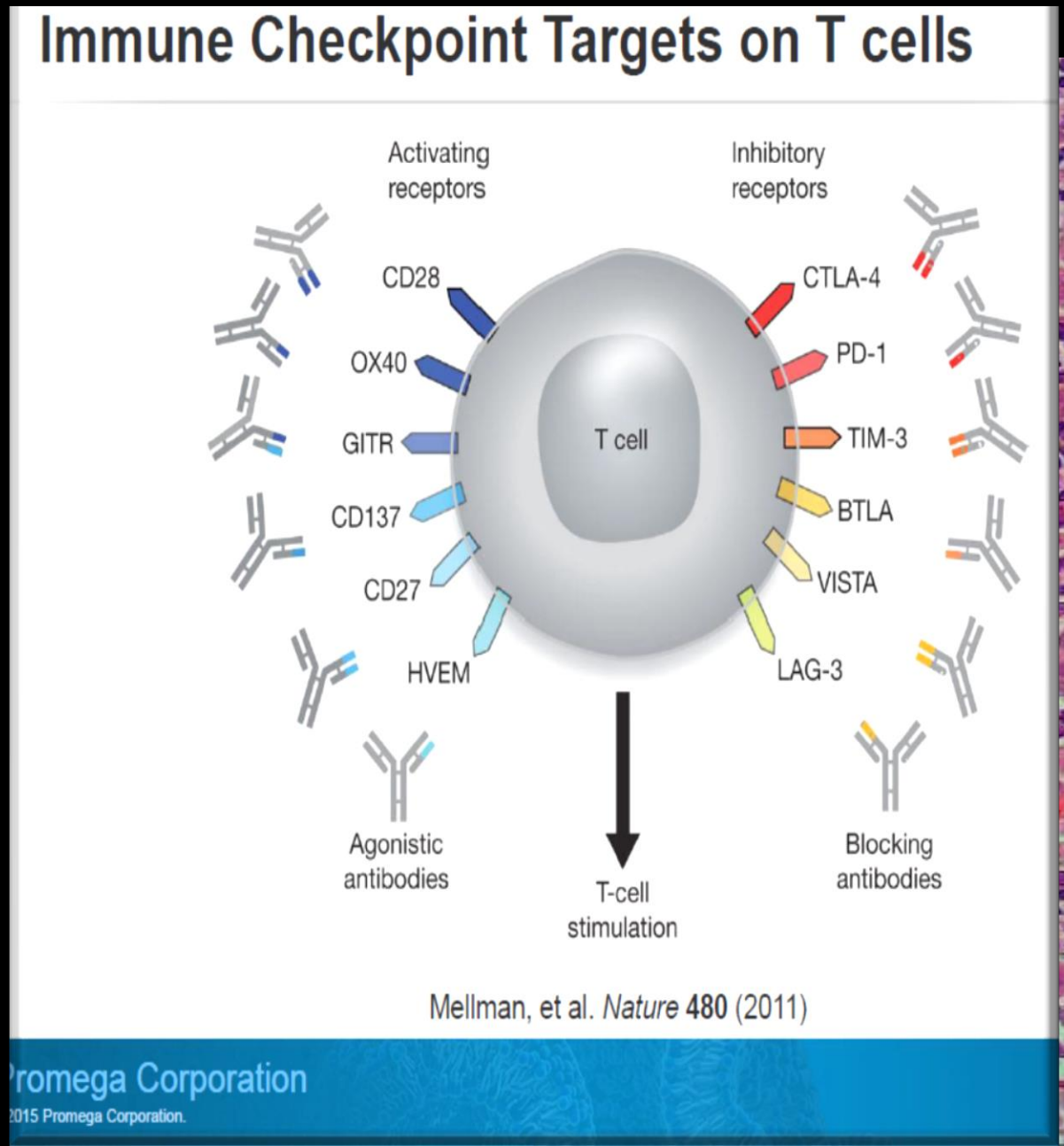
Angiogenesis inhibitors

Immunotherapies

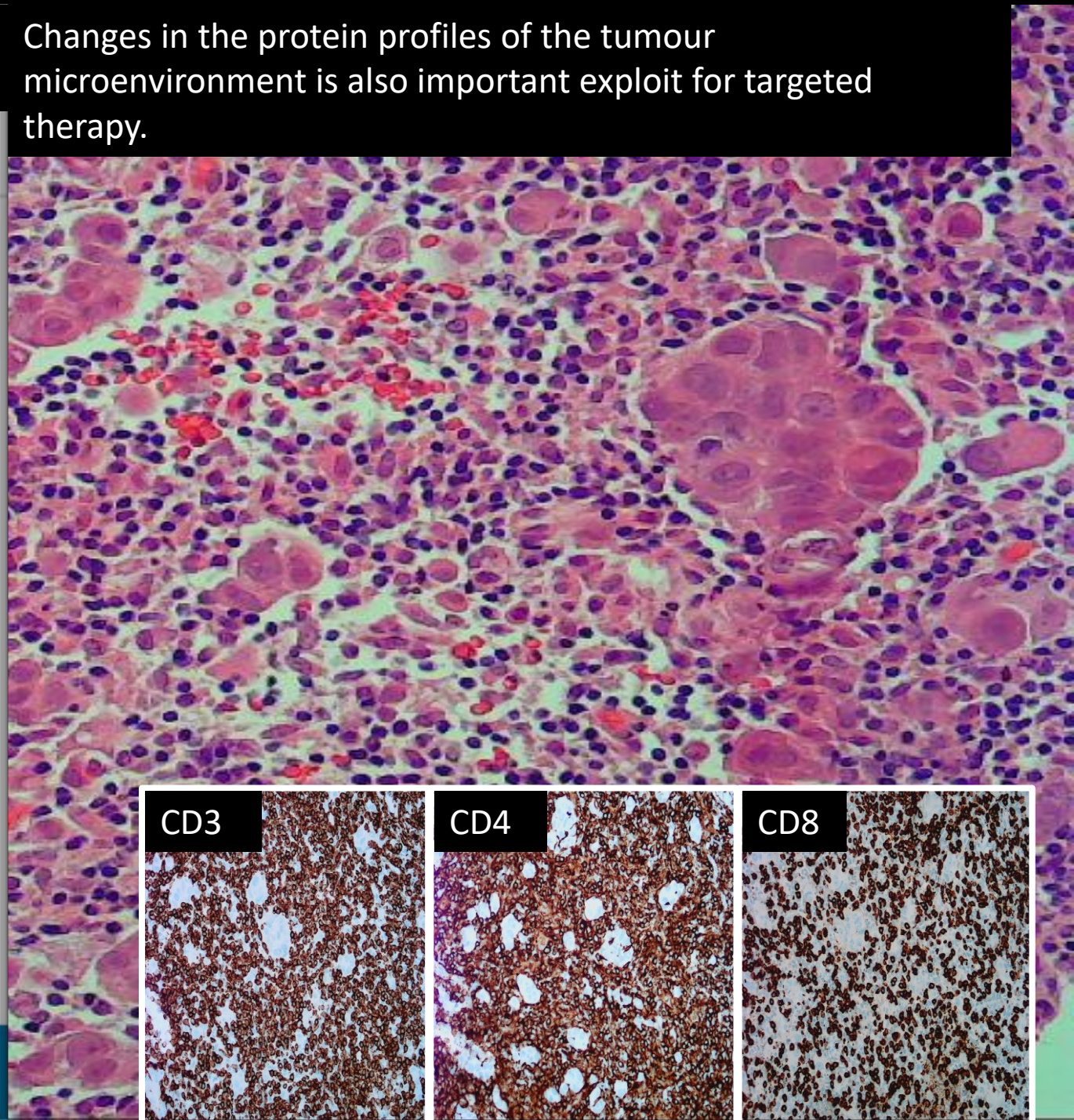
Monoclonal antibodies that deliver toxic molecules

Cancer vaccines and gene therapy

Tumour infiltrating cytotoxic T- lymphocytes (cancer immunotherapy). Immune checkpoint inhibitors



Changes in the protein profiles of the tumour microenvironment is also important exploit for targeted therapy.



BREAST PATHOLOGY

LYMPHORETICULAR PATHOLOGY

CLINICAL HISTORY/PICTURE, LABORATORY / RADIOLOGICAL FINDINGS

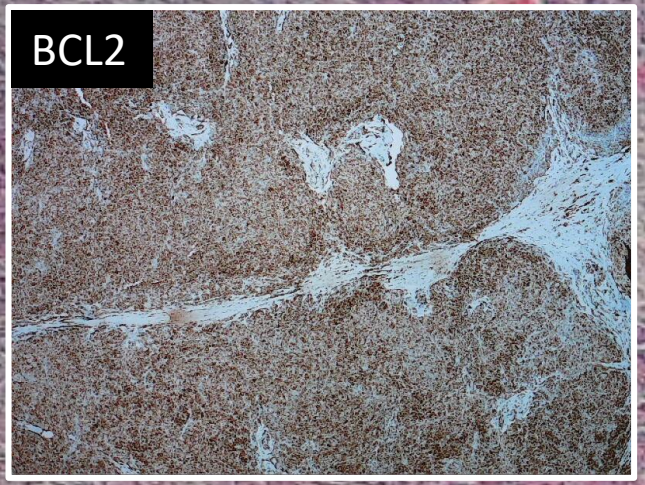
MORPHOLOGY, HISTOCHEMISTRY,IMMUNOHISTOCHEMISTRY,EM

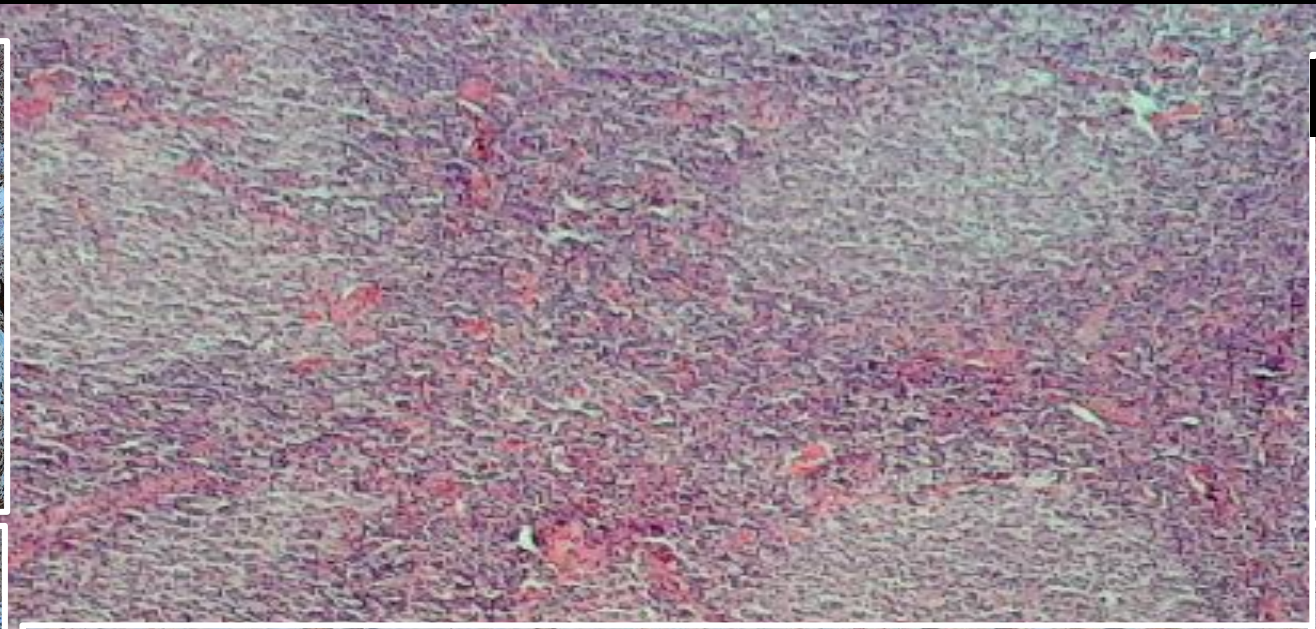
Her2, TOP 2A , MULTI GENE AMPLIFICATION.

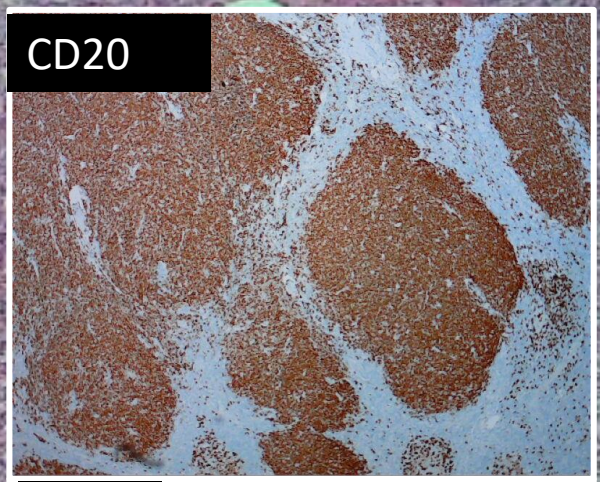
MOLECULAR PATHOLOGY

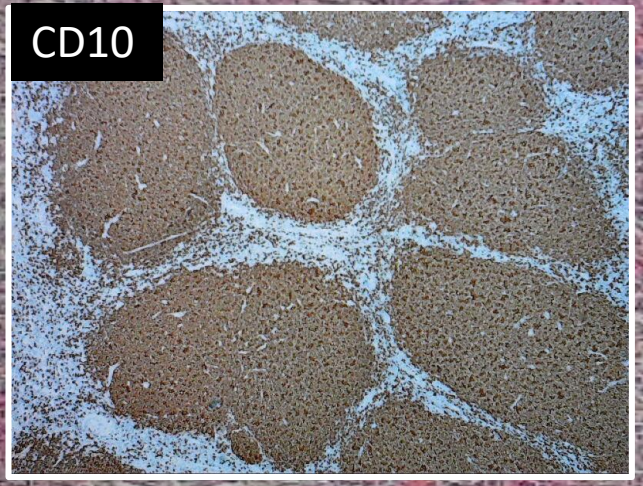
B – CELL Ig H GENE & T- CELL RECEPTOR GENE REARRANGMENT
MULTIPLE TRANSLOCATIONS

INTEGRATED DIAGNOSTIC, PROGNOSTIC AND PREDICTIVE (THERAPEUTIC) PATHOLOGY REPORT

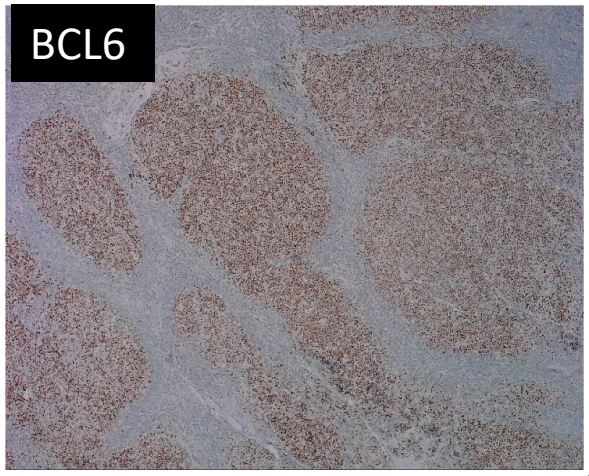

BCL2

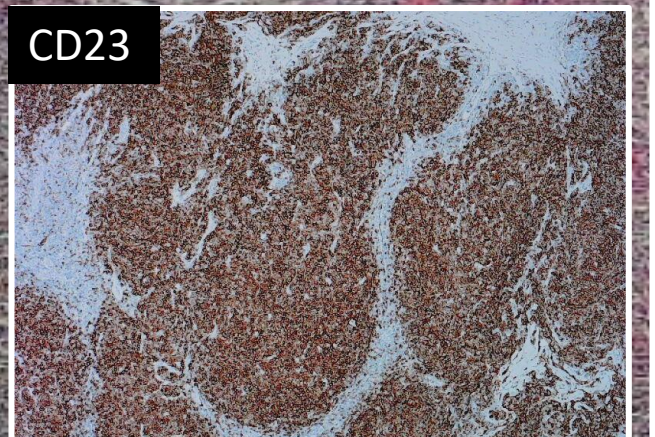


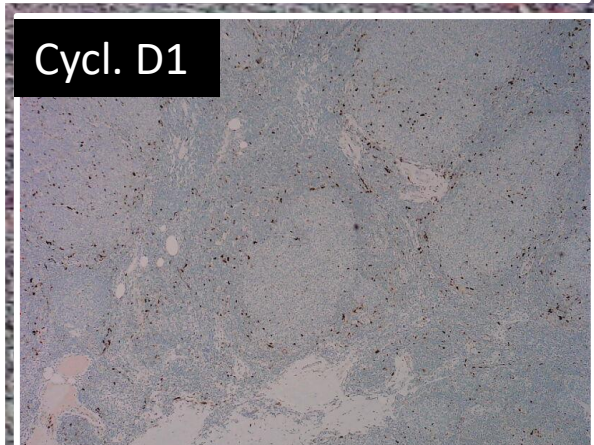

CD20


CD10

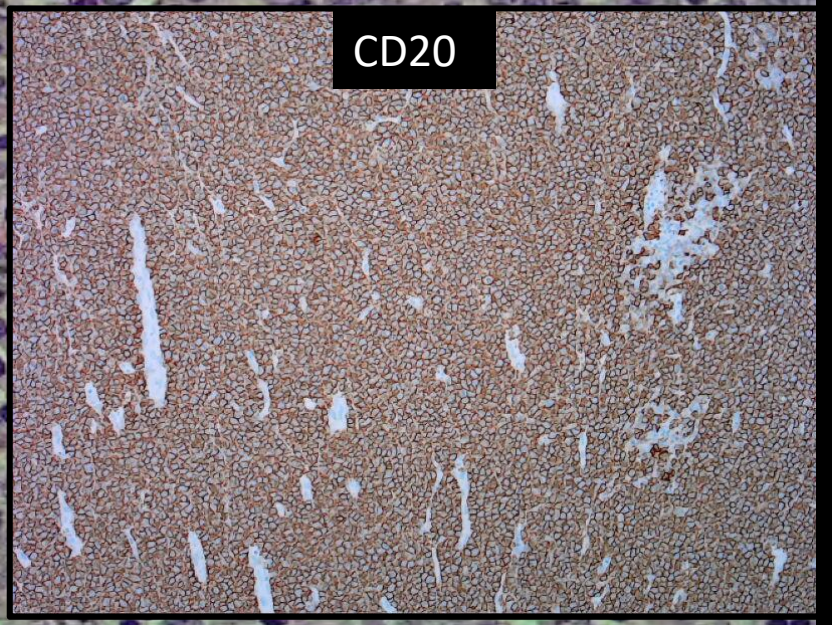

CD5
Diagnosis:-
B-Cell Follicle centre Lymphoma, Follicular Grade 2
Week CD5 +Ve
FISH Bcl2 - Rearranged t(14;18)(q32;q21)


BCL6


CD23


Cycl. D1

Lf. Tonsil



Diagnosis:-
Diffuse Large B Cell Lymphoma NOS
FISH for MYC (8q24) normal (no MYC/Bcl2,Bcl6, MYC/IgH translocation)

Molecular Profiling of Lung NSCCa.

LUNG ADENOCARCINOMA MOLECULAR CLASSIFICATION

- EGFR WILDE TYPE
  GEFITINB, ERLOTINIB
  NON RESPONSIVE
- EGFR MUTANT TYPE
  GEFITINIB, ERLOTINIB
  RESPONSIVE

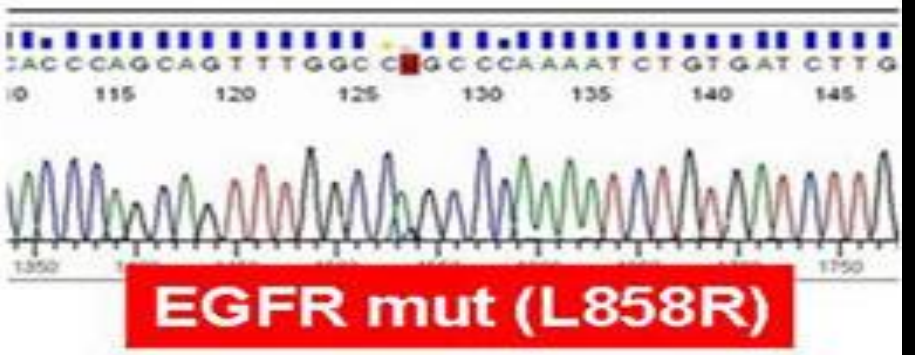
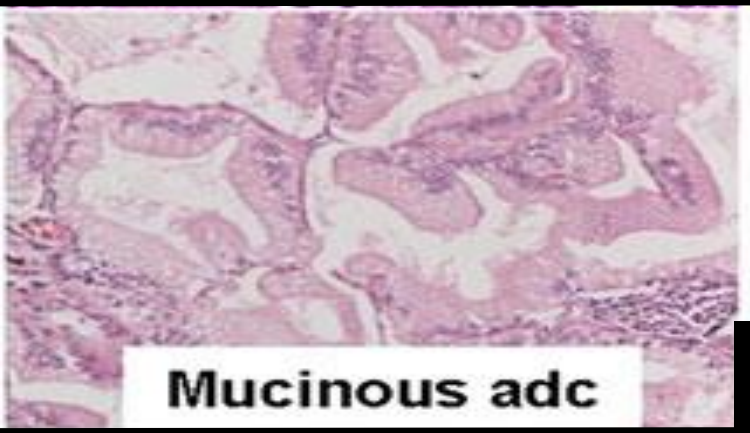
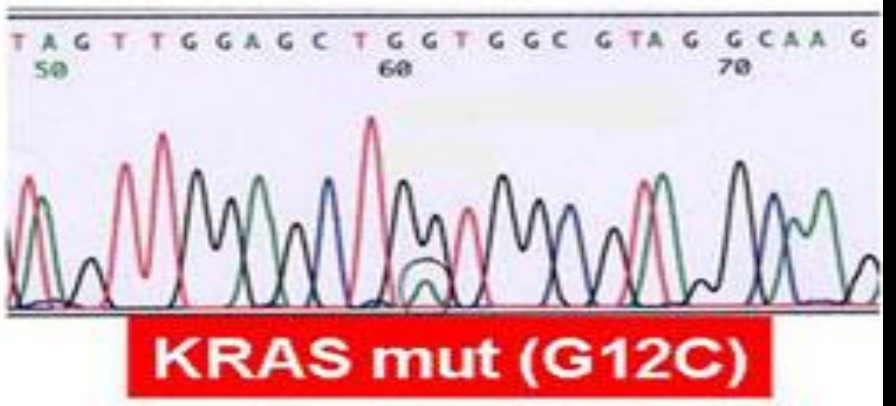
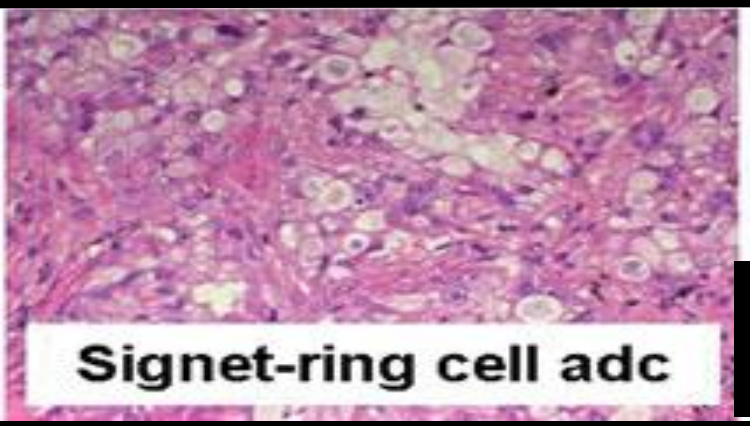
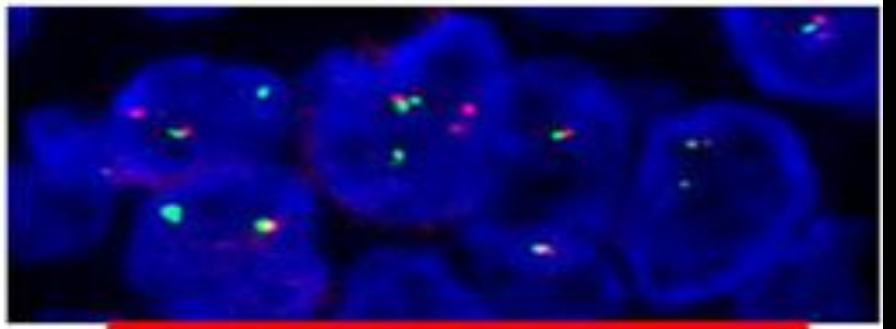
LUNG ADENOCARCINOMA MOLECULAR CLASSIFICATION

- ALK1 + ve
  AK1-EML4 gene fusion
  Tyrosine kinase inhibitors Responsive
- ALK1 – ve
  Tyrosine kinase inhibitors Non- Responsive

Common driver gene mutations in lung cancer

- EGFR mutations
- KRAS mutations
- EML4-ALK Rearrangements
- MET mutation

These 4 mutations are generally mutually exclusive and are only rarely seen in the same tumour.

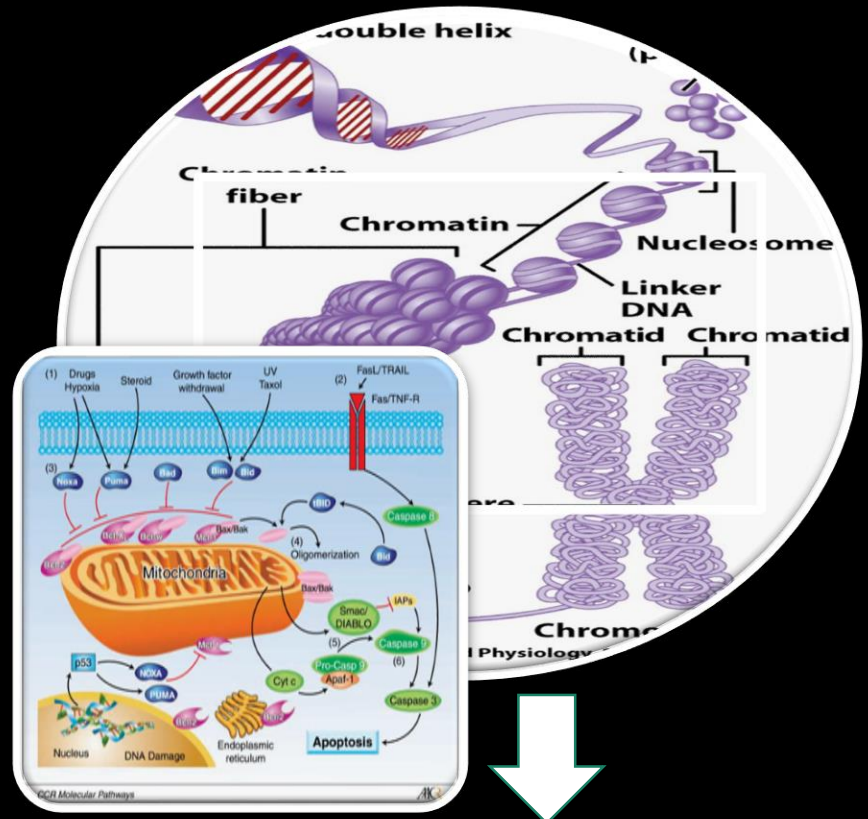
Morphology	Immunohistochemistry	Molecular features
 Papillary adc	TTF-1 + / p63 -	 EGFR mut (L858R)
	Diagnosis:- **TTF1 –VE EGFR Mut. Papillary Adenocarcinoma**	
 Mucinous adc	TTF-1 - / p63 -	 KRAS mut (G12C)
	Diagnosis:- **TTF1 –VE KRAS Mut. Mucinous Adenocarcinoma**	
 Signet-ring cell adc	TTF-1 + / p63 +	 ALK rearrangement
	Diagnosis:- **TTF1 +VE P63 +VE ALK1 Rearranged Signet – Ring cell Adenocarcinoma**	

Virchows Arch

NGS

(New Generation Sequencing)

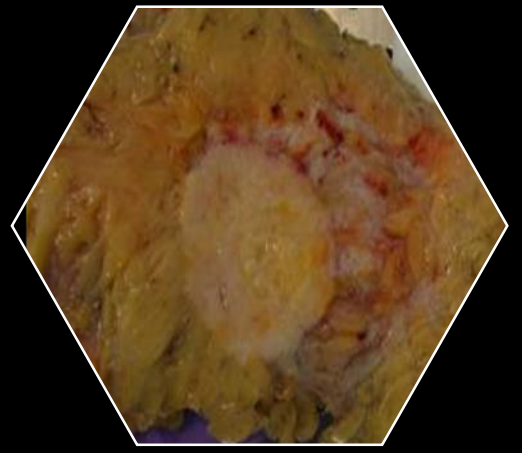
Sequencing DNA and RNA much more quickly and cheaply



WGS

(Whole Genome Sequencing)

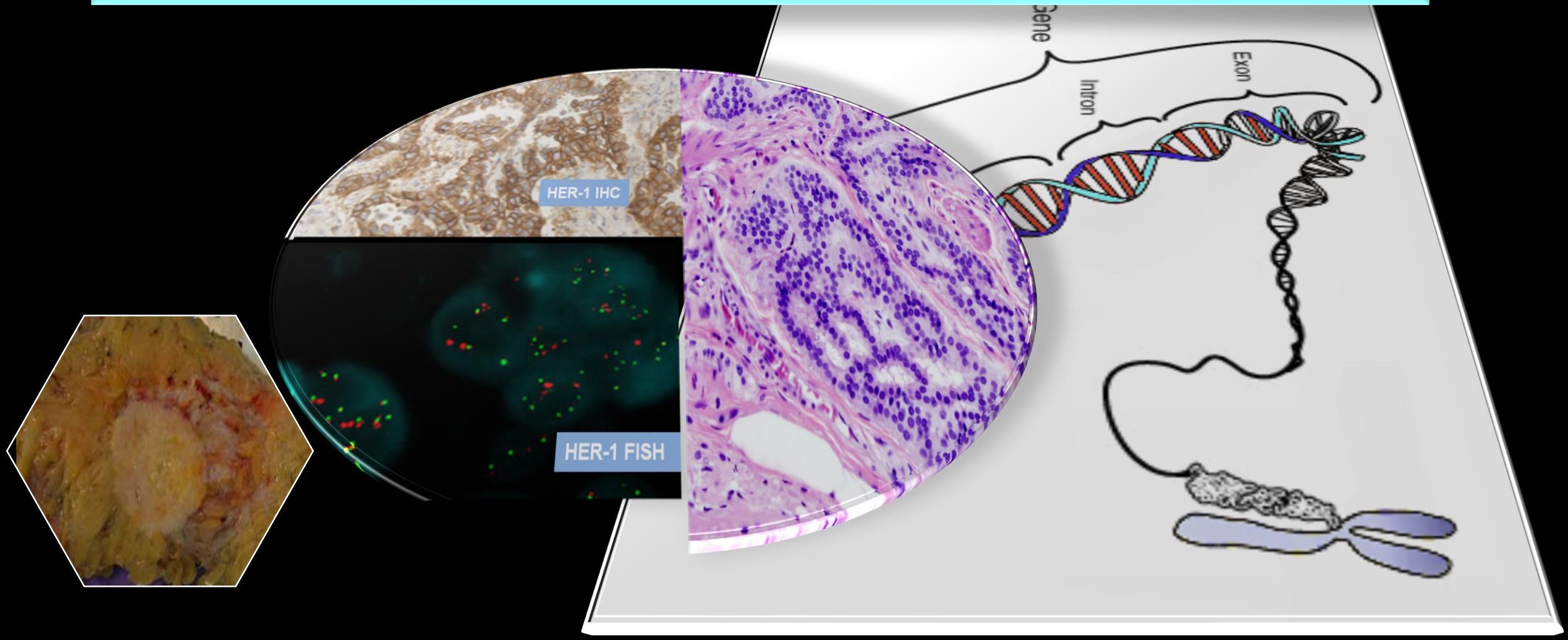
Full genome sequencing process of determining the complete DNA sequence of organism's genome at a single time (greater than 95%), which includes nuclear DNA and Mitochoria DNA, rapidly becoming a tool for evolutionary biology of disease process as such may lay the foundation for prediction of disease susceptibility and drug response (personalised medicine)

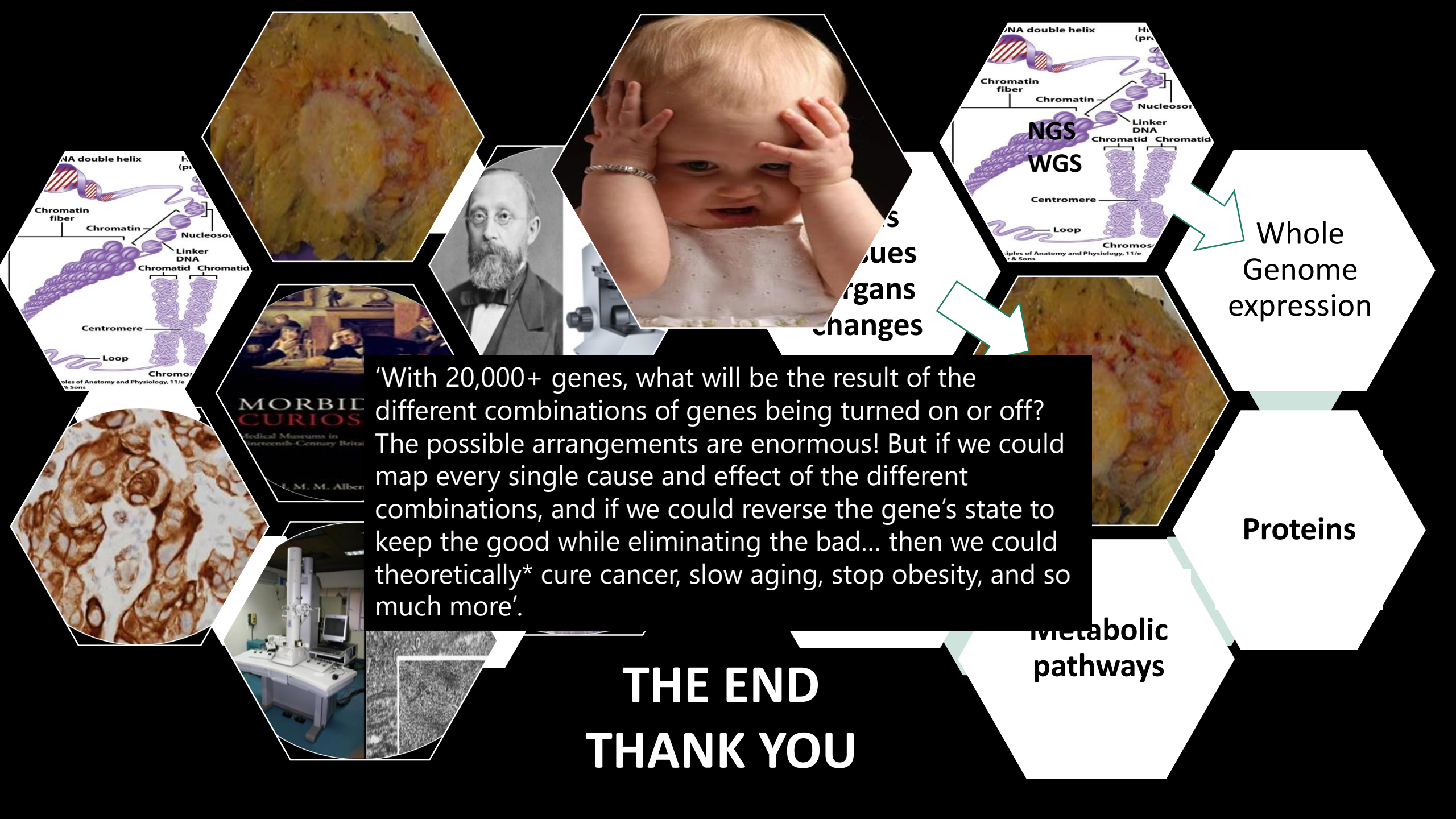


Advances of new molecular diagnostic tests NGS,WGS and including ‘liquid biopsy’ would gives us the opportunity of using molecular fingerprint panels which would inform us of the health and disease status of individual well before clinically manifest disease .

Primary healthcare provision may have to provide a new service based around prevention.

‘Primary Care Pathologist’



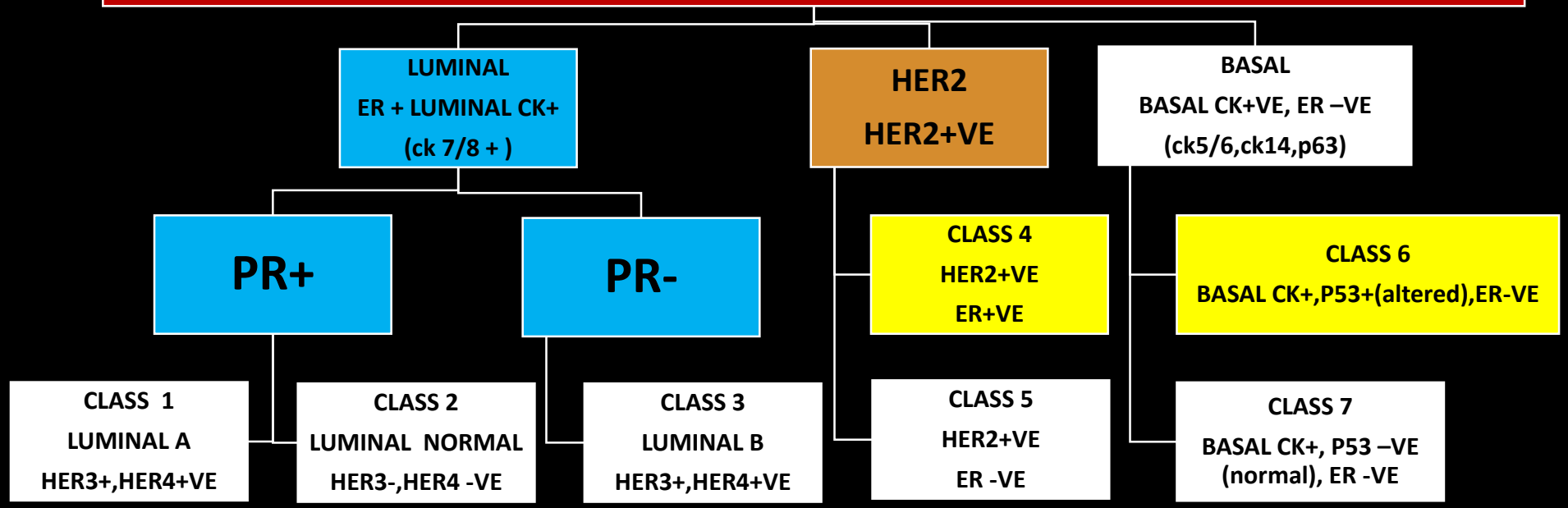

NGS
WGS
Whole Genome expression
Proteins
Metabolic pathways
'With 20,000+ genes, what will be the result of the different combinations of genes being turned on or off? The possible arrangements are enormous! But if we could map every single cause and effect of the different combinations, and if we could reverse the gene's state to keep the good while eliminating the bad... then we could theoretically* cure cancer, slow aging, stop obesity, and so much more'.
THE END
THANK YOU

Summary

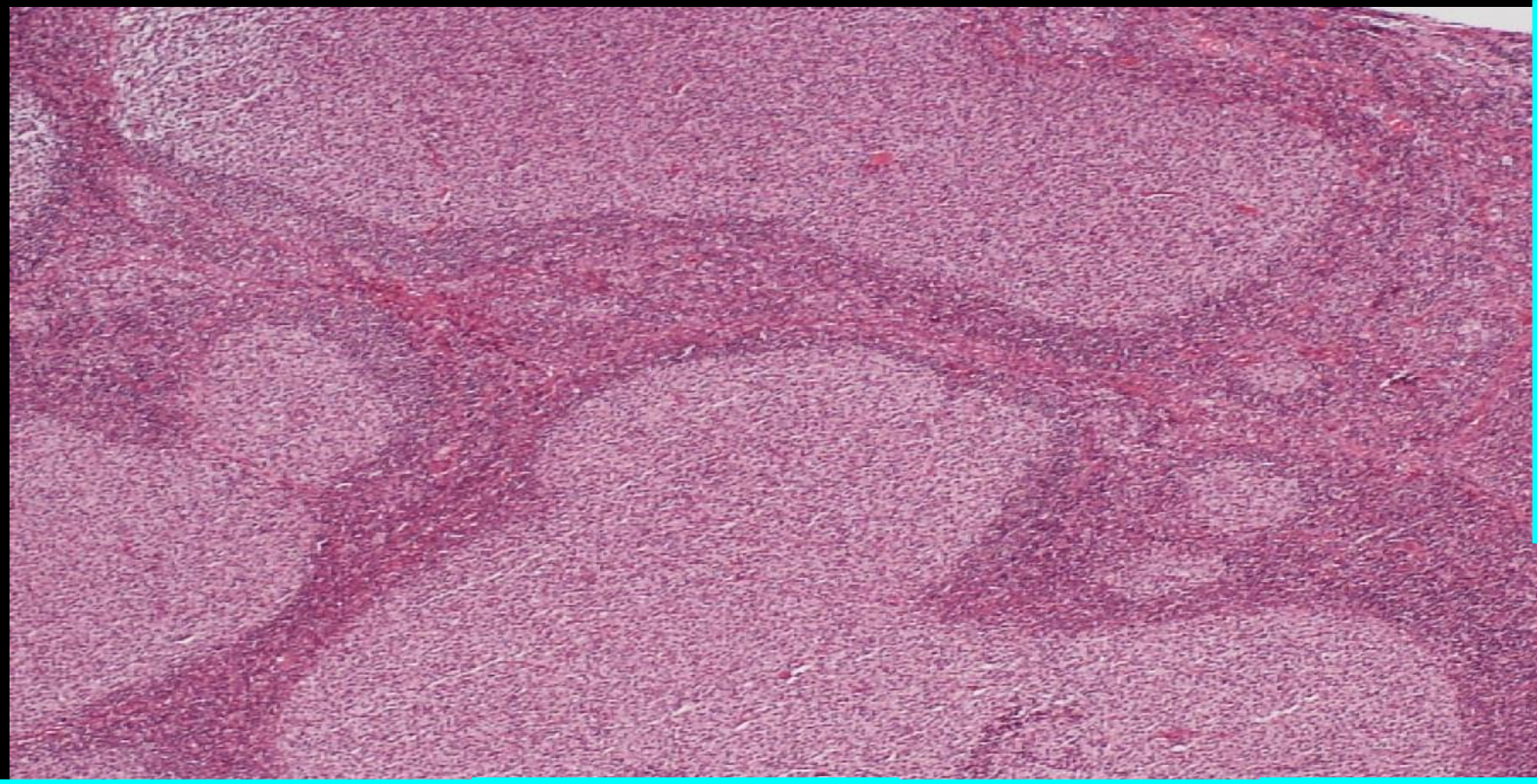
- **Genome sequencing revolution has the potential reshape provision of health care and make individualised medicine a reality , Pathologist should accrue the skills necessary to interpret and act upon the whole genome data and pathology training curriculum have to reflect this.**
- **Pathologists and biomedical scientists should be proactive in developing their practices and grab the opportunities offered by advances in new technologies shifting the emphasis from morphological criteria only to individualised and therapeutically driven molecularly based pathology, make the best use of a new clinically validated data to issue an integrated diagnostic, prognostic and predictive(therapeutic) pathology report and be part of important decision making regarding patient care.**
- **There is a valid point to argue for having one stope molecular histopathology laboratories covering conventional histopathology and tumour molecular pathology under one roof.**
- ***'Primary care pathologist'* pathologists should realise an era of primary care pathologist analysing genomic information and advising primary care physician on risk management and disease prevention is a realistic future prospect .Pathologists must decide how to participate in this activity and how to partner with other health care professionals such as genetic counsellors to develop direct patient interactions as part of the new practice of primary care pathology.**

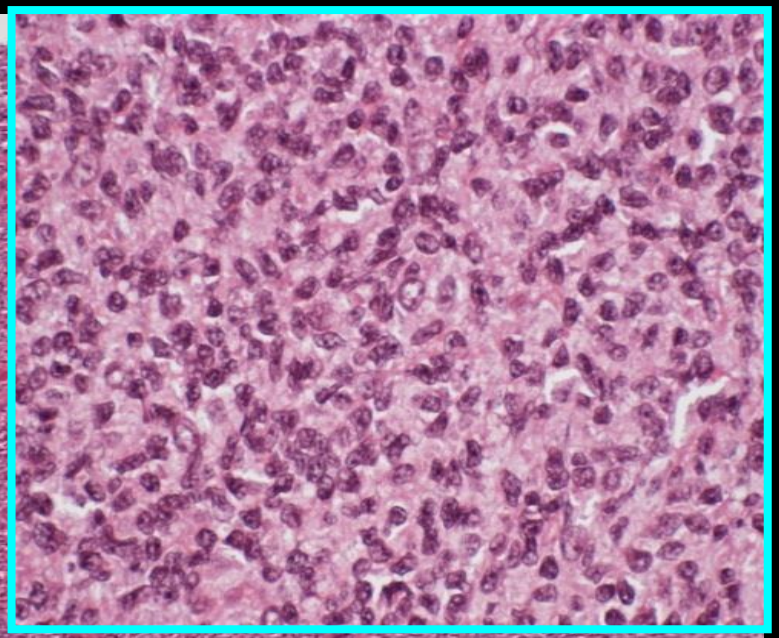
‘With 20,000+ genes, what will be the result of the different combinations of genes being turned on or off? The possible arrangements are enormous! But if we could map every single cause and effect of the different combinations, and if we could reverse the gene’s state to keep the good while eliminating the bad… then we could theoretically* cure cancer, slow aging, stop obesity, and so much more’.

BREAST CARCINOMA PHENOTYPIC TYPES – NPI + GROUPS

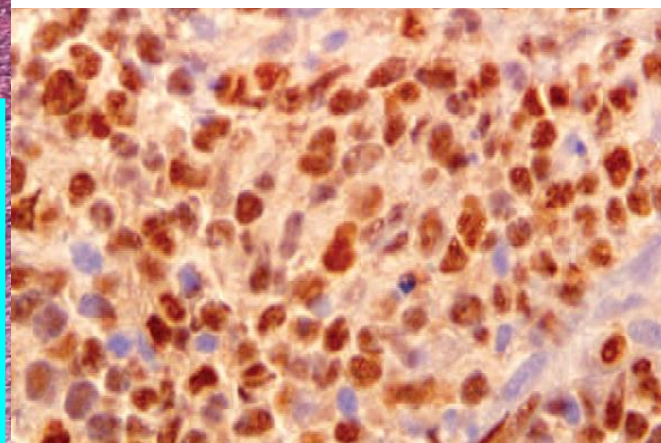


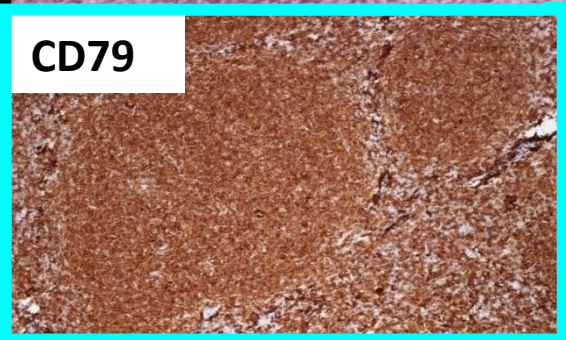
F.69Y.LT. AXILLARY LYMPHADENOPATHY, 5.7 cm. NODE

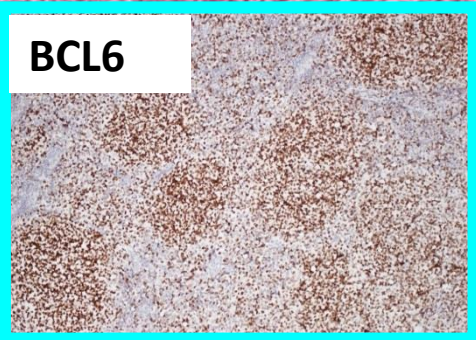


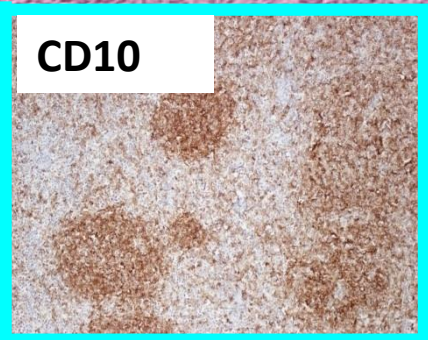


LMO2 (LIM-only transcription factor 2) IN FOLLICULAR L.









- The aims to identify the changes in the tumour protein landscape due to aberrant signalling pathways as a manifestation of tumour genomic alteration.
- These proteins regulate metastatic potential, cell proliferation survival and immune evasion.
- Most also represent the targets for therapeutic intervention in cancer
- Translating these changes to immunohistochemical/in situ hybridisation etc surrogates and its interpretation is the prime remit of histopathology.