Objectives

- Appreciate the nomenclature of tumours/neoplasms
- Understand the differences between benign and malignant neoplasms
- Understand the evolution of precursor lesions to invasive cancer
- Identify general risk factors of cancer
- Appreciate the spectrum of carcinogenic agents and their mechanisms of action
- Understand the molecular basis of cancer, including the 8 hallmarks and 2 enabling characteristics
- Describe how cancer-associated genes can be dysregulated
- Appreciate the various clinical manifestations of neoplasms
- Understand the principles of grading and staging and techniques used for prognostication and predicting response to therapy

Outline

I. Nomenclature and Characteristics of Benign vs Malignant Tumours

a. **Definitions and Morphologic descriptors:** *Tumour, Neoplasms, Polyp, Papilloma*
b. **Nomenclature (based on cell lineage)**
c. **Comparison of Benign vs Malignant neoplasms:** *Degree of differentiation, Presence of local invasion, Presence of metastasis (distant spread)*
d. **Precursor lesions:** *Hyperplasia, Metaplasia, Dysplasia, Carcinoma in-situ*

II. Epidemiology of Cancer

a. **Cancer Incidence and Mortality**
b. **Risk factors – Environmental:** *Infectious agents, Smoking, Alcohol consumption, Diet etc.*
c. **Risk factors – Host:** *Age, Acquired predisposing conditions (including precursor lesions), Genetic predisposition and Interactions between environmental and inherited factors*

III. Molecular Basis of Cancer

a. **Definitions and Mechanisms involved in Carcinogenesis:** *Driver mutations, Initiating mutations, Gene classes affected by cancer-causing mutations (proto-oncogenes, tumour-suppressor genes), Passenger mutations*
b. **Cellular and Molecular Hallmarks of Cancer:** *(1) Self-sufficiency in growth signals, (2) Insensitivity to growth-inhibitory signals, (3) Altered cellular metabolism, (4) Evasion of apoptosis, (5) Limitless replicative potential (immortality), (6) Sustained angiogenesis, (7) Ability to invade and metastasize, (8) Ability to evade the host immune response. These are facilitated by sources of Genomic instability and Cancer-promoting inflammation*
c. **Mechanisms of Dysregulation of Cancer-associated genes:** *Gene mutations, Chromosomal changes, Epigenetic changes, Noncoding RNAs*

IV. Carcinogenic Agents

a. **Chemical Carcinogenesis:** *Direct vs Indirect-acting*
b. **Radiation Carcinogenesis:** *UV rays, Ionizing radiation*

c. **Microbial Carcinogenesis:** *HLTV-1, HPV, EBV, HBV/HCV, Helicobacter pylori*

V. Clinical Aspects of Neoplasia

a. **Clinical Manifestations:** *Local effects, Hormonal effects, Cachexia, Paraneoplastic syndromes*
b. **Grading and Staging of Tumours**
c. **Laboratory Diagnosis of Tumours:** *Tissue sampling methods, Frozen section, Ancillary techniques (Immunohistochemistry, Flow Cytometry, Molecular Diagnostics), Molecular profiling, Tumour markers, Circulating tumour cells and cell-free DNA/RNA*

References

Kumar V, Abbas A, Aster J. *Robbins & Cotran Pathologic Basis of Disease*. 10th ed.

***Note**: Pathweb Study Notes are based on the key topics covered in the lectures in the Yong Loo Lin School of Medicine, as well as additional topics covered in major texts. For more comprehensive discussion on specific pathology topics, readers are advised to refer to the recommended texts in your respective courses.*

I. NOMENCLATURE AND CHARACTERISTICS OF BENIGN VS MALIGNANT TUMOURS

Tumour = Swelling (initially because of inflammation, now equated with neoplasm)

Neoplasm ("New growth") = an abnormal mass of tissue, the growth of which **exceeds** and is **uncoordinated** with that of normal tissue, and **persists** in the same excessive manner after cessation of the stimuli which evoked the change

- Arises from **excessive proliferation** of **cells** (and **stroma)** arising from a genetic disorder of cell growth triggered by acquired or inherited mutations affecting a single cell and its clonal progeny (i.e. **unregulated cell growth**)
- Neoplastic cells constitute the tumour parenchyma, while the stroma is reactive and comprises connective tissue and blood vessels with infiltrating inflammatory cells. The growth and spread of tumour is dependent on its stroma
- Tumours can have varying proportions of cells to stroma → tumours with scant connective tissue are soft and fleshy, while those with stromal desmoplasia (i.e. formation of abundant collagenous stroma stimulated by the tumour cells) are hard or scirrhous

Benign tumours = tumours that **remain localized** at their site of origin. Generally amenable to surgical removal, no/low risk of mortality (conditional on tumour site e.g. benign brain tumours can cause significant morbidity and occasional fatalities)

- **Hamartoma** = **disorganized** mass composed of cells indigenous to the involved tissue. Once thought to be a developmental malformation but now considered benign neoplasm (most hamartomas recently discovered to have clonal chromosomal aberrations acquired through somatic mutation) vs
- **Choristoma** = heterotopic (misplaced) rest of cells e.g. **normally organized** pancreatic tissue in the stomach. **Not considered a neoplasm**

Malignant tumours (aka **cancer**) = tumours that have **potential to invade** and destroy adjacent structures, and **spread to distant sites (metastasize**). Patient outcome depends on stage at which it is diagnosed and treated, and responsiveness to available treatment

Morphologic descriptors:

- **Polyp** = grossly visible projection above a mucosal surface (regardless of benign or malignant, epithelial or mesenchymal proliferation)
- **Papilloma** = benign epithelial neoplasm forming finger-like or warty projections from epithelial surfaces

Nomenclature depends on neoplastic cell lineage and tumour behaviour (benign vs malignant):

Cell lineage	Benign	Malignant
Mesenchymal origin	***(-oma)***	***(-sarcoma)***
Connective tissue (fibroblasts) (fat)	Fibroma Lipoma	Fibrosarcoma Liposarcoma
Vessels	Haemangioma	Angiosarcoma
Muscle (smooth) (striated)	Leiomyoma Rhadomyoma	Leiomyosarcoma Rhabdomyosarcoma
Epithelial origin	***(adenoma / papilloma)***	***(carcinoma)***
Squamous	Squamous cell papilloma	Squamous cell carcinoma
Epithelial lining of glands/ducts	Adenoma	Adenocarcinoma
Liver (hepatocytes)	Hepatocellular adenoma	Hepatocellular carcinoma
Urinary tract (urothelium)	Papilloma	Urothelial carcinoma
Melanocytes	Naevus	Malignant melanoma
Blood cells		
Haematopoietic cells		Leukaemia
Lymphoid cells		Lymphoma
Mixed tumours *(tumours with more than one line of differentiation)*		
Salivary gland	Pleomorphic adenoma	Carcinosarcoma
Teratogenous tumours *(tumours from more than one germ cell layer)*		
Totipotential cells in gonads / embryonal rests	Mature teratoma	Immature teratoma Teratocarcinoma

Comparison of benign vs malignant neoplasms

3 main distinguishing characteristics: 1. Degree of differentiation, 2. Local invasiveness, 3. Presence of distant spread. The latter two are most reliable:

(I) Degree of differentiation

- **Differentiation** = extent to which neoplastic parenchymal cells **morphologically** and **functionally** resemble the corresponding normal parenchymal cells; **Anaplasia** = lack of differentiation
- Benign tumours are generally well-differentiated
- Malignant tumours exhibit a spectrum of well-, moderately- and poorly-differentiated, and may exhibit **morphologic alterations** reflecting their potential for aggressive behaviour:
    - **Pleomorphism** = variation in cell size and shape. Highly pleomorphic tumours may have tumour giant cells (i.e. much larger tumour cells with one or more abnormal nuclei)
    - **Abnormal nuclear morphology** = nuclear enlargement with increased nuclear:cytoplasmic ratio, irregular nuclear contours, coarse chromatin with hyperchromasia (more darkly stained than usual), prominent nucleoli
    - **Mitoses** = increased numbers of cells in mitoses, reflecting their high proliferative rate. Note! Normal or reactive lesions with high cell turnover e.g. gut mucosal epithelium also have increased numbers of mitoses. However, bizarre or atypical mitoses (e.g. tripolar forms) are always abnormal and a stronger indicator of malignancy

- **Loss of cellular/nuclear polarity** = loss of orientation of tumour cells with respect to each other or supporting structures e.g. basement membrane
- **Necrosis** = often a reflection of a rapidly growing tumour outgrowing its vascular supply, leading to areas of ischaemia

- Tumours that retain the **functional capabilities** of their normal counterparts are more likely well-differentiated e.g. keratin formation in squamous cell carcinomas, hormone secretion in well-differentiated neuroendocrine tumours. Poorly-differentiated or undifferentiated tumours not only lose their specialized functional activities, but may also acquire express new unanticipated proteins e.g. fetal proteins, proteins normally found in other cell types

(II) Presence of local invasion

- Besides development of metastasis, invasiveness is the next most reliable discriminator of benign vs malignant tumours

Benign tumours	Malignant tumours
Lack capacity of local invasion, usually slow-growing and respects anatomical boundaries	Demonstrate progressive invasion and destruction of surrounding tissue; growth may be slow or rapid
May have a surrounding capsule i.e. a rim of compressed fibrous tissue, forming a tissue plane facilitating surgical excision. Appear discrete and movable	Poorly demarcated from its surroundings – surgical excision requires margin clearance. Appear 'fixed' (non-movable)
Exception: Haemangiomas (benign vascular neoplasm) are often unencapsulated - may be difficult to excise completely	Caveat: Some slowly-expanding malignant tumours may develop an apparent fibrous capsule with 'pushing' borders, but histologic examination will show tumour cell infiltration

(III) Presence of metastasis (distant spread)

- Tumour spread to sites that are physically discontinuous with the primary tumour – usually taken as unequivocal indicator of malignancy
- The invasiveness of cancers allows them to penetrate blood vessels, lymphatics and body cavities, which provides opportunity for spread. All malignant tumours have the potential to metastasize but it is a complex process with the risk of metastases dependent on both tumour and host factors (many exceptions exist; not necessarily correlating with invasiveness) e.g. lack of differentiation, aggressive local invasion, large tumour size
- Metastatic spread strongly decreases the possibility of cure and is thus important to prevent
- Pathways of cancer spread:
    1. **Direct seeding of body cavities and surfaces**: When a malignant neoplasm penetrates into a natural 'open field' lacking physical barriers e.g. peritoneal cavity. Commonly seen in ovarian carcinomas
    2. **Lymphatic spread**: Most common pathway for initial dissemination (carcinomas, sometimes sarcomas). Tumours do not contain functional lymphatic vessels, but tumour cells can invades and spread through lymphatic vessels located at the tumour periphery. Pattern of spread follows the **natural routes of lymphatic drainage** e.g. breast cancers in the upper outer quadrant spread to axillary lymph nodes first. **Sentinel lymph node** = first node in a regional lymphatic basin that receives lymph flow from the primary tumour; can be

identified and examined to guide need for further surgical therapy e.g. axillary clearance while minimizing morbidity.
Note 1: "skip metastasis" may occur, possibly because microscopic metastases are missed or due to variation in normal patterns of lymphatic drainage.
Note 2: Enlarged lymph nodes do not always harbour metastases – lymphoid hyperplasia may also occur as immune response to tumour cells or antigens in draining lymph nodes (when tumour cells are destroyed by a tumour-specific immune response within the node)

3. **Haematogenous spread**: Typical of sarcomas, also carcinomas. Presence of vascular invasion (usually involving thin-walled veins vs thicker-walled arteries) at the tumour site is associated with higher risk of haematogenous spread. Pattern of vascular metastasis depends on type of vessel invasion (venous invasion → first capillary bed e.g. all portal drainage to liver, all caval blood to lung), tumour site (e.g. tumours near vertebral column like thyroid gland → paravertebral plexus) and tumour type (e.g. renal cell carcinomas have a propensity for growth within large veins, although it may not be accompanied by widespread metastasis)

Precursor lesions: localized morphologic changes that identify a field of epithelium at increased risk for malignant transformation e.g. hyperplasia, metaplasia or dysplasia

- **Hyperplasia** = often results from chronic exposure to trophic factors e.g. endometrial hyperplasia from sustained estrogenic stimulation
- **Metaplasia** = replacement of one cell type with another. Nearly always associated with tissue damage, repair and regeneration, with the replacing cell type better suited to alteration of the local environment e.g. squamous to columnar epithelium in Barrett oesophagus due to increased acidity
- **Dysplasia** = 'disordered growth', used to describe epithelial cells displaying morphologic alterations of malignancy to varying degrees (e.g. pleomorphism, nuclear atypia, architectural disorder) but without invasion. May be a precursor to malignant transformation, but **does not always progress to cancer** and may even regress if the inciting cause is removed.
    - **Carcinoma in-situ (CIS)** = severe form of dysplasia involving full epithelial thickness but with still intact basement membrane (i.e. non-invasive). Unless treated, CIS has a high probability of progression to invasive cancer, although it takes time (probably for the accumulation of mutations to occur)
- Although some benign neoplasms can undergo malignant transformation (e.g. colonic villous adenoma), most benign tumours transform rarely (e.g. uterine leiomyomas) or not at all (e.g. lipoma). The reason for this difference is still unknown, although one possibility is that benign tumours at high risk for malignant transformation possess the cancer-enabling property of genomic instability whereas other benign tumours do not

II. EPIDEMIOLOGY OF CANCER

Cancer incidence and mortality

- Major cause of mortality (~1 in 6 of all deaths worldwide)
    - Most common tumours in men: prostate, lung, colon/rectum
    - Most common tumours in women: breast, lung, colon/rectum
- Incidence varies with geography (mainly from different environmental exposures), age, race and genetic background. Race is not a discrete biologic variable but can define groups at risk for certain cancers
- In higher income countries, the age-adjusted cancer death rate increased significantly in both men and women from 1950-1990, but has decreased by ~10-20% since 1990 while cancer incidence rates have stabilized, largely due to decreased use of tobacco products for lung cancers, and improved detection/screening and treatment for colorectal, breast, prostate and cervical cancers

Risk factors - Environmental

Environmental influences (known or unknown) are the dominant risk factor for most cancers:

- **Infectious agents**: ~15% of all cancers worldwide are caused directly or indirectly by infectious agents (with greater burden in the developing world) *(see "section IV. Carcinogenic agents")*
- **Smoking**: (particularly cigarettes) implicated in cancer of ~90% of lung cancers as well as the mouth, pharynx, larynx, oesophagus, pancreas, bladder
- **Alcohol consumption**: increases risk of carcinoma of oropharynx (excluding lip), larynx, oesophagus (synergistic effect with tobacco use) and (via alcoholic cirrhosis) hepatocellular carcinoma
- **Diet**: precise dietary factors remain debatable but has been attributed as a cause of geographic variation in incidence of colorectal, prostate and breast carcinoma
- **Obesity**: overweight individuals have higher death rates from cancer
- **Reproductive history**: lifelong cumulative exposure to estrogen stimulation including pregnancies (particularly if unopposed by progesterone) increases risk of breast and endometrial cancer
- **Environmental carcinogens**: may be in the ambient environment, workplace or in food e.g. UV rays, asbestos, arsenic (in well water), certain medications

Risk factors - Host

Age

- Cancer incidence rises with age - most carcinomas occur in adults older than 55 years of age. Likely due to accumulation of somatic mutations that accompanies cell aging, and a decline in immune competence in older people
- Cancer also affects children, but the types of cancer (e.g. leukemia, central nervous system neoplasms) are different from adults (carcinomas), partly because paediatric cancers are more likely to be caused by inherited mutations (particularly in tumour suppressor genes) and less likely from exposure to environmental carcinogens

Acquired predisposing conditions

- **Chronic inflammatory disorders:** both infectious and non-infectious chronic inflammatory disorders increase cancer risk, mostly carcinomas but also mesothelioma and certain lymphomas. Pathogenesis: these disorders cause tissue injury, with (1) resulting compensatory cell proliferation to repair the damage, (2) increased pool of tissue stem cells which may be particularly susceptible to transformation, and (3) activated immune cells that produce reactive oxygen species (ROS) that may damage DNA and inflammatory mediators that promote cell survival even in the face of genomic damage. Diagnosis and treatment of the cause e.g. eradication of Helicobacter pylori organisms with antibiotics can help prevent possible future development of gastric carcinoma
- **Precursor lesions:** localized morphologic changes that identify a field of epithelium at increased risk for malignant transformation e.g. hyperplasia, metaplasia or dysplasia (*see above section*)
- **Immunodeficiency states:** predispose to virus-induced cancers (particularly T-cell immunodeficiencies), possibly because these individuals have higher incidence of chronic infection with viruses

Genetic predisposition and Interactions between environmental and inherited factors

- Familial cancers may result from hereditary germline mutations in tumour suppressor genes
- Sporadic cancers are largely attributable to environmental factors or acquired predisposing conditions, but may also have an inherited component despite the lack of family history as the interaction between hereditary and nonhereditary contributions are often complex, especially when tumour development depends on multiple genes. Non-genetic factors can influence the risk of cancer development even in cancers with a well-defined inherited component (e.g. BRCA carriers), while genetic factors can alter the likelihood of cancers primarily induced by environmental carcinogens (e.g. polymorphisms in enzymes affect the conversion of procarcinogens to active carcinogens)

III. MOLECULAR BASIS OF CANCER

Carcinogenesis depends on the **stepwise accumulation of mutations over time, resulting in nonlethal genetic damage**. These genomic alterations result in several phenotypic attributes (aka **cancer hallmarks**) e.g. excessive growth, local invasiveness and ability to metastasize, that are complementary to produce a fully malignant tumour

- **Driver mutations** = mutations that contribute to the acquisition of cancer hallmarks
- The initial damage (or mutation) may be caused by environmental factors, inherited or spontaneous / random. This is also known as the **"initiating mutation".** However, a single mutation is not sufficient for malignant transformation; the "initiated" cell needs to acquire additional driver mutations that each contribute to the development of cancer
- Cancer-causing mutations usually affect 4 gene classes:
    1. **Proto-oncogenes** (growth-promoting): **'gain of function'** mutations cause an increase in the normal function of the encoded gene product or an entirely new function that is oncogenic. These effects are *dominant* over a normal copy of the same gene

2. **Tumour suppressor genes** (growth-inhibiting): **'loss of function'** mutations usually require both alleles to be damaged before the effects are seen and thus behave in a *recessive* manner at the cellular level (Knudson's "two-hit" hypothesis – in familial cases, children inherit one defective copy (first hit) and thus only need spontaneous somatic mutation in the other normal allele (second hit) to develop disease, while in sporadic cases, both normal alleles must undergo somatic mutation in the same cell (two hits), which is much less probable. The increased risk of cancer in familial cases is inherited as an *autosomal dominant* trait). Exception: some genes require both alleles for normal function, therefore loss of only one (**haploinsufficiency**) causes significant loss of quantity of the encoded protein to affect cell proliferation and survival
3. **Genes that regulate programmed cell death** (apoptosis): include gain-of-function mutations in genes encoding proteins that suppress apoptosis and loss-of-function mutations in genes that encode proteins promoting cell death, thereby enhancing cell survival
4. **Genes that are responsible for DNA repair**: cells with impaired recognition and repair of nonlethal genetic damage will acquire mutations at a faster rate (i.e. a **mutator phenotype characterized by genomic instability**), thereby facilitating carcinogenesis

- Loss-of-function mutations in genes that maintain genomic integrity appear to be a common early step, especially in solid tumours. Genomic instability facilitates acquisition of additional driver mutations as well as "**passenger mutations**" i.e. mutations that have no phenotypic consequence. As passenger mutations are much more common that driver mutations, tumour cells will have many more passenger mutations by the time it acquires all the driver mutations needed for malignant behaviour
    - Besides driver and passenger mutations, there may be mutations in other genes that indirectly contribute to tumorigenesis e.g. by interfering with host immune responses, altering stromal interactions
- A single precursor cell with these mutations undergoes clonal expansion and forms a tumour (i.e. **tumours are clonal in origin)**. Since DNA alterations are heritable, all cells within the tumour share the same set of mutations that were present at the moment of transformation
- However, as the tumour expands, individual tumour cells acquire additional mutations at random (especially in tumours with driver mutations conferring a mutator phenotype). The tumour thus **evolves to become genetically heterogeneous** by the time they become clinically evident (generally ~1g or about 10^9 cells). The different tumour subclones compete for access to nutrients and microenvironmental niches under the pressure of Darwinian selection (survival of the fittest); the most "fit" subclones will then dominate the tumour mass. The tumour thus **progresses to become more aggressive** over time
    - Therapy also acts as a selection pressure on tumours; tumours that recur after therapy are almost always resistant to the same treatment presumably because therapy selects subclones that can survive despite treatment
- In addition to DNA mutations, **epigenetic modifications** which determine gene expression can contribute to carcinogenesis and include DNA methylation (tends to silence gene expression e.g. of tumour suppressor genes) and histone modification (can either enhance or dampen gene

expression). Epigenetic modifications, unlike DNA mutations, are potentially reversible by drugs that inhibit DNA-modifying or histone-modifying factors

Cellular and molecular hallmarks of cancer

The genomic and epigenomic alterations of cancers result in eight fundamental changes in cell biology, which are considered **hallmarks of cancer**. **Genomic instability** and **cancer-promoting inflammation** can accelerate the acquisition of these changes. **Loss of normal cell cycle control** is central to malignant transformation, and at least one of four key regulators of the cell cycle (p16/INK4a, cyclin D1, CDK4, RB) is dysregulated in most human cancers.

1. **Self-sufficiency in growth signals**: tumours can proliferate without external stimuli (usually due to oncogene activation)
   - Normal physiologic growth factor-induced signaling involves (1) binding of a growth factor to its specific receptor; (2) transient limited activation of the growth factor receptor which in turn activates several cytoplasmic signal-transducing proteins; (3) transmission of the transduced signal to the nucleus via additional cytoplasmic proteins / signaling cascade; (4) induction and activation of transcription factors and epigenetic alterations that initiate and sustain DNA transcription; (5) expression of genes and encoded factors that result in cell division, as well as other genes that support cell survival and metabolic alterations needed for optimal growth
   - **Oncogenes** = mutated / overexpressed versions of normally regulated growth-promoting genes (proto-oncogenes) that cause excessive cell growth even in the absence of growth factors and other external cues (i.e. the expressed oncoproteins are **constitutively active**)
   - Aberrations can occur in multiple signaling pathways, and affect the different components:

Category	Proto-oncogene product	Description
Growth factors	FGF PDGF	Most growth factors function in a **paracrine fashion** i.e. they are made by one cell type and act on a neighbouring cell of a different type with the appropriate growth factor receptor. Some cancer cells can form **autocrine loops** i.e. they produce the same growth factor to which they are responsive
Growth factor receptors	Receptor tyrosine kinase e.g. EGF-receptors, PDGF receptor	Receptor tyrosine kinases are transmembrane proteins with an extracellular growth factor-binding domain and a cytoplasmic tyrosine kinase domain. The oncogenic versions of these receptors have **mutations that lead to constitutive growth factor-independent tyrosine kinase activity**, including point mutations (e.g. ***EGFR*** in lung cancer), gene rearrangements (e.g. ***ALK*** in lung cancer) and amplifications (e.g. ***HER2*** in breast cancer). Targeted therapies that aim to inhibit the enzymatic activity of these growth factor receptors have proven effective to some extent, but are usually not curative in advanced cancers (which have probably acquired additional mutations that sidestep the drug effects e.g. by preventing EGFR inhibitors from binding to the receptor)
Signal transduction proteins	GTP-binding (G) proteins KRAS, GNAS	**Activating mutations** in downstream components e.g. RAS are often mutually exclusive with mutations in receptor tyrosine kinases, implying that RAS activity can completely substitute for tyrosine kinase activity. ***RAS***

	RAS signal transduction e.g. BRAF	Point mutations of the *RAS* family genes are the most common type of proto-oncogene abnormality in human tumours. RAS proteins are membrane-associated small G proteins that bind GTP/GDP, with intrinsic GTPase activity (which can be augmented by GTPase-activating proteins - GAPs) to terminate signal transduction. Several distinct *RAS* point mutations can reduce the GTPase activity of the RAS protein, resulting in a permanently activated GTP-bound form causing continuous pro-growth signal transduction. Loss-of-function mutations in GAPs have a similar consequence (e.g. neurofibromin 1 encoded by *NF1* gene). Development of RAS inhibitors has been largely unsuccessful as it requires restoration of a missing enzymatic activity (GTPase activity). **MAPK and PI3K/AKT cascades** These lie downstream of RAS and consist of a series of kinases that can have oncogenic gain-of-function mutations (factors near the top of the cascade are most likely mutated): - *BRAF*: serine/threonine protein kinase. Activating mutations stimulate downstream kinases and ultimately activate transcription factors. Treatment with BRAF inhibitors has great response but only in tumours with *BRAF* mutations - *PI3K*: activating mutations stimulate downstream kinases e.g. AKT, which phosphorylates >150 proteins and is a major signaling node. PI3K is negatively regulated by PTEN (a tumour suppressor)
	Non-receptor tyrosine kinase	**Nonreceptor tyrosine kinases e.g. ABL tyrosine kinase** These tyrosine kinases normally localize to the cytoplasm or nucleus instead of the membrane, but also appear able to activate the same signaling pathways as receptor tyrosine kinases. 2. Mutations tends to be chromosomal translocations / rearrangements that create fusion genes encoding constitutively active tyrosine kinases e.g. in chronic myeloid leukemia, translocation of *ABL* gene on chr 9 to chr 22 where it fuses with *BCR* gene results in a chimeric BCR-ABL protein with constitutive tyrosine kinase activity. BCR-ABL inhibitors are highly effectively in CML as a result of **oncogene addiction** (whereby tumour cells are highly dependent on the activity of one oncoprotein despite accumulation of mutations in other cancer-associated genes). However, treatment only inhibits the proliferative component of the tumour but does not lead to cure; rare CML "**stem-like cells**" harbouring BCR-ABL fusion gene persist, apparently because these cells do not require BCR-ABL signals for their survival and thus are resistant to therapeutic targeting. 3. There can also be activating point mutations in nonreceptor tyrosine kinases that remove the function of negative autoregulatory domains e.g. JAK2 (involved in the JAK/STAT signaling pathway) in myeloproliferative neoplasms
Nuclear regulatory proteins	Transcription factors e.g. MYC	Transcription factors regulate the expression of pro-growth genes and cyclins ***MYC*** is the most commonly affected transcription factor of this class in cancer. The *MYC* proto-oncogene is expressed in virtually all eukaryotic cells, and is an immediate early response gene that is rapidly and transiently induced by RAS/MAPK signaling following growth factor stimulation. MYC has multiple actions: (1) MYC activates expression of many genes involved in cell growth and is considered a master transcriptional regulator of cell growth e.g. D cyclins (for cell cycle progression), rRNA genes and processing (for

		ribosome assembly and protein synthesis), genes involved in metabolic reprogramming (2) MYC can upregulate telomerase expression in some cases (3) MYC can act with other transcription factors to reprogram somatic cells into pluripotent stem cells, possibly contributing to cancer cell "stemness" MYC can be upregulated through multiple mechanisms, including genetic alterations of MYC itself (e.g. translocation in B- and T-cell lymphomas, MYC gene amplification), oncogenic mutations of upstream signaling pathways (e.g. RAS/MAPK) that increase MYC transcription, enhance MYC mRNA translation, and/or stabilizing MYC protein. Several single nucleotide polymorphisms (SNPs) associated with increased risk for cancers e.g. prostate/ovarian carcinoma lie within enhancer elements that flank MYC and appear to stimulate higher levels of MYC RNA expression in response to growth-promoting signals
Cell cycle regulators	Cyclin D1 CDK4	**Cyclin-dependent kinases** (CDKs) are activated by binding to **cyclins**; the CDK-cyclin complexes phosphorylate crucial target proteins that drive cell cycle progression. **CDK inhibitors** silence CDKs and exert negative control over the cell cycle. The 2 main cell cycle checkpoints (G_1/S and G_2/M transition) are tightly regulated by a balance of growth-promoting and growth-suppressing proteins, as well as sensors of DNA damage which transmit signals to arrest cell cycle progression and possibly initiate apoptosis. Defects in G_1/S checkpoint lead to dysregulated growth and may also impair DNA repair, creating a "mutator" phenotype, and include: 4. Gain-of-function mutations in D cyclin genes and CDK4 (which normally form a complex that phosphorylate RB) 5. Loss-of-function mutations in genes that inhibit G_1/S progression e.g. CDK inhibitors like *p16/INK4 (CDKN2A)* (which normally binds cyclin D-CDK4 and promotes the inhibitory effects of RB) or *p14/ARF* (normally increases p53 levels by inhibiting MDM2 activity)

2. **Insensitivity to growth-inhibitory signals**: tumours do not respond to molecules that inhibit cell proliferation (usually due to inactivation of tumour suppressor genes)
   - **Tumour suppressor proteins** control checkpoints that prevent uncontrolled growth and oppose any of the various hallmarks of cancer. They may cause **growth inhibition** in the presence of genotoxic stress, resulting in cellular quiescence and eventually apoptosis, or cause cells to undergo **differentiation** (enter a postmitotic state without replicative potential)
   - Tumour suppressor proteins form components of signaling pathways and cell cycle regulators (similar to proto-oncogenes), as well as regulators of cellular responses to DNA damage:

Category	***Tumour suppressor gene* (product)**	**Description**
Inhibitors of mitogenic signaling pathways	*APC* (chr 5q21) *NF1*	**APC** (adenomatous polyposis coli) protein is a tumour suppressor that downregulates growth-promoting signaling pathways. Germline loss-of-function is associated with **familial adenomatous polyposis**, an AD disorder in which people with one inherited mutated allele develops thousands of adenomatous polyps in the colon in their 10s-20s, of which at least one

		inevitably progresses to colon cancer after acquiring additional mutations. 70-80% of sporadic colon cancers and adenomas also show acquired defects involving both APC genes. APC is a component of the **WNT signaling pathway** (important for controlling cellular growth and differentiation during embryonic development). In the absence of WNT signaling, APC participates in a 'destruction complex' involved in the proteasomal degradation of β-catenin. Loss of APC function with disruption of the destruction complex thus functions similarly to continuous WNT signaling, whereby β-catenin is stabilized and translocates from the cytoplasm to the nucleus, where it forms a transcription activation complex with a DNA-binding factor TCF to promote the growth of colonic epithelial cells. In colon tumours with normal APC genes, they often harbour β-catenin mutations that prevent its APC-dependent destruction, thereby leading to increased expression of β-catenin-dependent target genes. β-catenin is thus itself a proto-oncoprotein. Dysregulation of APC/β-catenin pathway can also be seen in other tumours e.g. Hepatocellular carcinoma
Inhibitors of cell cycle progression	*RB* *CDKN2A* (p16/INK4a, p14/ARF)	**RB** is a key negative regulator of the **G1/S cell cycle transition**. RB normally binds and inhibits E2F transcription factors needed to drive gene expression for progression to S phase. Can be directly or indirectly inactivated in most human cancers via: 1. Loss-of-function mutations involving both RB alleles 2. Shift from its active hypophosphorylated state to the inactive hyperphosphorylated state, e.g. through gain-of-function mutations that upregulate CDK/cyclin D activity or loss-of-function mutations affecting CDK inhibitors (e.g. p16/INK4a) 3. Transforming viral proteins e.g. E7 protein from high-risk types of HPV can bind to hypophosphorylated RB at the same site that E2F usually binds, therefore freeing E2F transcription factors to cause cell cycle progression ***CDKN2A*** gene encodes 2 protein products: 1. **p16/INK4a**: cyclin-dependent kinase inhibitor which blocks CDK4/cyclin-D mediated phosphorylation of RB. P16 may also be important in induction of cellular senescence 2. **p14/ARF**: inhibits MDM2, thereby preventing destruction of p53
Inhibitors of pro-growth programs of metabolism and angiogenesis	*VHL* (chr 3p)	**VHL** is a component of a protein complex that covalently links ubiquitin chains to specific protein substrates, thereby promoting their proteasomal degradation. Germline loss-of-function mutations are associated with hereditary renal cell carcinoma and other tumours; somatic mutations also occur in some sporadic RCC. VHL helps regulate cellular responses to oxygen levels; loss of function mutations in VHL prevent the ubiquitination and degradation of HIF1α (hypoxia-inducible transcription factor 1α) even under normoxic conditions, therefore resulting in increased levels of angiogenic growth factors (VEGF) and changes in cellular metabolism that favour growth (e.g. PDGF, GLUT1)
Inhibitors of invasion and metastasis	*CDH1* (E-cadherin)	**E-cadherin** is a cell surface protein that maintains intercellular adhesiveness, and binds to β-catenin. Germline loss-of-function of *CDH1* is associated with familial gastric carcinoma, and some sporadic gastric carcinomas are also associated with loss of E-cadherin expression. Loss of cell-cell contact e.g. due to epithelial injury disrupts the E-cadherin-β-catenin interaction, allowing β-catenin to translocate to the nucleus and

		stimulate proliferation (similar to WNT signaling). Re-establishment of E-cadherin contacts e.g. as the wound heals leads to sequestration of β-catenin at the membrane and reduces proliferative signaling → 'contact-inhibited'. Loss of E-cadherin (e.g. via mutation) therefore leads to loss of contact inhibition and increases proliferation, and also allows cell dyshesion with subsequent local invasion or metastasis
Enablers of genomic stability	*TP53* (chr17p13.1)	***TP53*** (aka Guardian of the Genome) regulates cell cycle progression, DNA repair, cellular senescence and apoptosis – most frequently mutated gene in human cancers (usually acquired but rarely can be germline – Li-Fraumeni syndrome). Can be directly or indirectly inactivated via: 1. Loss-of-function mutations 2. Other mutations affecting proteins that regulate p53 function e.g. MDM2 (which normally ubiquinates p53, stimulating its degradation by the proteasome) 3. Transforming viral proteins e.g. E6 protein of high-risk HPV binds p53 and promotes its degradation p53 levels are usually very low in normal cells due to the inhibitory effects of MDM2. In cellular stress (e.g. DNA damage, shortened telomeres, hypoxia, excessive pro-growth signaling), p53 can be released via 2 major mechanisms (depending on the nature of the stress): (I) DNA damage and hypoxia: ATM or ATR detects DNA damage/hypoxia and stimulate the phosphorylation of p53 and MDM2, thereby disrupting the binding and degradation of p53 by MDM2 (II) "Oncogenic" stress: cellular stress caused by activation of oncoproteins e.g. RAS lead to increased expression of p14/ARF, which binds MDM2 and displaces p53 Once p53 accumulates to levels sufficient to activate the transcription of target genes with p53-binding regulatory levels, several outcomes are possible (likely dependent on the duration and level of p53 activation) which protect against neoplastic transformation: • **Transient p53-induced cell cycle arrest**: Transient. Occurs late in G1 phase, caused partly by p53-dependent transcription of CDKN1A (encoding p21). p21 (like p16) inhibits CDK4/D cyclin complexes, thereby maintaining RB in an active hypophosphorylated state and blocking progression of cells from G1 to S phase. This allows cells time to repair the DNA damage. p53 also induced proteins e.g. GADD45 to enhance DNA repair. Once DNA repair is successful, p53 levels will fall and release the cell cycle block • **p53-induced senescence**: Permanent cell cycle arrest. May be stimulated in response to variety of stresses e.g. unopposed oncogene signaling, hypoxia and shortened telomeres. Mechanism still unknown • **p53-induced apoptosis**: p53 directs the transcription of pro-apoptotic genes e.g. BAX, inducing cell death via the intrinsic (mitochondrial) pathway If p53 function is lost, DNA damage goes unrepaired and driver mutations can accumulate, facilitating neoplastic transformation. Tumours with p53 mutations also do not respond as much to radiation and chemotherapy compared to tumours with intact p53 function, as these 2 modalities work by inducing DNA damage and subsequent apoptosis. Furthermore, these tumours with defective p53 also acquire a mutator phenotype, with a higher chance of developing subclones resistant to therapy

3. **Altered cellular metabolism**:
   Tumour cells switch to aerobic glycolysis (**Warburg effect**) even in the presence of ample oxygen, thus allowing synthesis of macromolecules and organelles needed for rapid cell growth
   - Rapidly growing cells rely on aerobic glycolysis, characterized by high levels of glucose uptake and increased conversion of glucose to lactose (fermentation). This "glucose hunger" can be visualized on positron emission tomography (PET) scanning
   - Despite being seemingly inefficient (in terms of generation of ATP), aerobic glycolysis provides rapidly dividing cells with metabolic intermediates that are needed for synthesis of cellular components, which mitochondrial oxidative phosphorylation does not
   - This metabolic reprogramming in tumours is produced by signaling cascades downstream of growth factor receptors that are deregulated by mutations in oncogenes and tumour suppressor genes – therefore, aerobic glycolysis ceases in rapidly growing normal cells that no longer grow, while in cancer cells this metabolic reprogramming becomes fixed e.g. receptor tyrosine kinase/PI3K/AKT signaling upregulates the activity of glucose transporters and multiple glycolytic enzymes. Conversely, tumour supressors often inhibit metabolic pathways that support growth e.g. STK11 tumour suppressor, p53

   Tumours may also utilize **autophagy** to their advantage. Autophagy is a state of severe nutrient deficiency in which cells arrest their growth and cannibalise their own cell components for energy production. Tumour cells can accumulate mutations to avoid autophagy, or use autophagy to become "dormant" and avoid being killed by therapies that kill actively dividing cells.

   Some oncoproteins e.g. mutated isocitrate dehydrogenase (IDH) involved in the Krebs cycle may also cause the formation of high levels of "**oncometabolites**" e.g. 2-HG that alter the epigenome. The resulting abnormal methylation / histone modification thereby leads to oncogenic changes in gene expression.

4. **Evasion of apoptosis**: tumours are resistant to programmed cell death
   - Tumour cells face various stresses that can initiate the intrinsic (mitochondrial) pathway of apoptosis e.g. DNA damage, metabolic disturbances, hypoxia; hence there is strong incentive for tumour cells to evade apoptosis, usually by acquired mutations or changes in gene expression that affect the intrinsic pathway (rather than the extrinsic death receptor pathway)
   - Two major mechanisms are:
     1. **Loss of TP53 function**, thereby preventing upregulation of PUMA in response to DNA damage and other stresses. PUMA is a pro-apoptotic BH3-only protein that normally neutralizes anti-apoptotic proteins like BCL2
     2. **Overexpression of anti-apoptotic members of the BCL2 family**. Common event, e.g. in follicular lymphoma with a t(14;18) translocation that fuses the BCL2 gene to the transcriptionally active IgH gene. These tumours tend to be indolent as they arise through reduced cell death rather than rapid cellular proliferation

5. **Limitless replicative potential (immortality)**: tumours acquire stem cell-like properties that allow proliferation without cellular senescence and mitotic catastrophe. This occurs through:

a) **Evasion of senescence:** Most normal human cells divide 60-70 times, then permanently leave the cell cycle. This senescent state is presumably associated with upregulated p53 and INK4a/p16, which maintain RB in a hypophosphorylated state and thus induce cell cycle arrest. Many cancers have genetic and epigenetic alterations that disrupt this RB-dependent G1/S cell cycle checkpoint, thereby allowing evasion of senescence

b) **Evasion of mitotic crisis:** Cells that evade senescence are still not immortal. Each cell division results in the progressive shortening of telomeres and eventual exposed chromosome ends that are "sensed" as double-stranded DNA breaks. If the cells have functional p53, they undergo cell arrest and possibly apoptosis, but if p53 is dysfunctional, the nonhomologous end-joining pathway is activated and may join the ends of the 2 chromosomes. Subsequent mitosis results in true double-stranded DNA breaks. This repeated "bridge-fusion-breakage" cycle causes snowballing genomic damage, mitotic catastrophe and cell death. Proliferating cells that evade senescence and reactivate telomerase to restore their telomeres can survive. As these cells are likely to have suffered damage to oncogenes and tumour suppressor genes during crisis, they are at high risk for malignant transformation. Alternatively, cancer may arise from stem cells that express telomerase, or use another mechanism to main their telomeres (alternate lengthening of telomeres)

c) **Capacity for self-renewal:** Self-renewal means each time a stem cell divides, at least one of 2 daughter cells remains a stem cell (asymmetric division) or both daughter cells remain stem cells (symmetric division e.g. during embryogenesis). The non-stem cell daughter differentiates, losing "stemness" but gaining other functions, and eventually stop dividing and become senescent or undergo apoptosis. Many tissues that contain short-lived cells e.g. bone marrow and epithelial cells of the gastrointestinal tract have a resident population of tissue stem cells. By extrapolation, cancers also must have cancer stem cells since they are immortal and have limitless proliferative capacity. These cancer stem cells may potentially arise from transformation of tissue stem cells, or somatic cells that acquire "stemness". The presence of these stem cells limits the success of current anti-cancer therapies due to their low rate of cell division and expression of factors e.g. multiple drug resistance-1 (MDR1) that counteracts chemotherapy effects

6. **Sustained angiogenesis**: tumour cells need to establish a vascular supply for nutrients and oxygen and remove waste products to allow continued growth
   - Tumours otherwise cannot grow beyond 1-2 mm in size (the presumed maximal distance across which oxygen, nutrients and waste can diffuse from existing blood vessels)
   - Neoangiogenesis also stimulates the growth of adjacent tumour cells by secreting growth factors e.g. PDGF, insulin-like growth factors (IGFs), and permits ready access by tumour cells to this new leaky, dilated and haphazard vascular network, facilitating metastasis
   - Angiogenesis depends on the balance between angiogenesis promoters and inhibitors. Most tumours remain small and do not induce angiogenesis until an **angiogenic switch** occurs, which involves increased local production of angiogenic factors and/or loss of angiogenic inhibitors from tumour cells, infiltrating inflammatory cells, other tumour-associated stromal cells and the ECM. This may be facilitated by several mechanisms:

- Relative lack of oxygen due to hypoxia stabilized HIF1α, a transcription factor that activates transcription of pro-angiogenic cytokines VEGF and bFGF
- Driver mutations in certain tumour suppressors and oncogenes favour angiogenesis e.g. gain of function mutations in RAS or MYC upregulate VEGF production
- Proteases (from tumour or stromal cells) release bFGF from the ECM, but can also release anti-angiogenic factors e.g. angiostatin

o Several therapeutic agents have been developed that block angiogenesis e.g. bevacizumab (a VEGF inhibitor), and can prolong survival but are not curative

7. **Ability to invade and metastasize**: most cancer deaths and morbidity are due to metastases. Local invasion is a prerequisite but does not always result in distant spread, likely due to the complexity of the metastatic cascade that involves interaction of tumour cells with different types of host cells and factors. At each step, the breakaway tumour cells also need to evade immune defenses and adapt to a different microenvironment. The steps include:
   a) **Invasion of Extracellular Matrix (ECM)**: To metastasize, tumour cells must breach the underlying basement membrane, traverse the interstitial connective tissue and penetrate the vascular basement membrane to gain access to the circulation. Invasion of the ECM is an active process in which tumour cells interact with the ECM, stromal cells, immune cells and endothelial cells:
      i. **Dissociation of tumour cells:** usually due to alterations in intercellular adhesion molecules e.g. E-cadherin. In many epithelial cancers, it is hypothesized that transient silencing of E-cad expression occurs during **epithelial-mesenchymal transition** (EMT), with concomitant upregulation of mesenchymal markers (e.g. vimentin and smooth muscle actin) to favour the development of a promigratory phenotype
      ii. **Degradation of the basement membrane and interstitial connective tissue**: tumour cells can secrete proteolytic enzymes or induce stromal cells to do so. Many tumours overexpress proteases e.g. matrix metalloproteinases (MMPs) that remodel the basement membrane and connective tissue, and also release factors that contribute to the malignant behaviour of the tumour e.g. angiogenic and growth-promoting factors
      iii. **Alteration in tumour-ECM interactions:** tumour cells show complex changes in the expression of integrins (transmembrane proteins that participate in cell-cell and cell-ECM adhesion). In normal epithelial cells, integrins are restricted to the basal aspect of the cell, helping to maintain cell polarity to the basement membrane. Loss of adhesion normally induces apoptosis, but free tumour cells express other integrins that mitigate this by transmitting signals to promote cell survival. Alteration of ECM itself can also generate novel sites that bind to receptors on tumour cells and stimulate migration
      iv. **Migration of tumour cells through the degraded basement membranes and zones of matrix proteolysis**: this involves many receptors and signaling pathways that affect the actin cytoskeleton e.g. tumour cell-derived cytokines which act as autocrine motility factors, cleave products of matrix components and stromal cell-derived paracrine factors that stimulate motility. Cells need to attach to the matrix at the leading edge, detach from the matrix at the trailing edge and contact the actin cytoskeleton to move forward

b) **Vascular dissemination, homing and colonization:**

i. Circulating tumour cells that **clump as multicellular aggregates in the blood are more likely than single cells to establish metastases** and can be achieved by tumour cell-tumour cell and tumour cell-blood components (particularly platelets) interactions. Tumour cells can also express substances to encourage fibrin deposition and further stabilization of tumour emboli. This presumably is advantageous in allowing the tumour cells to arrest within the capillary bed, and also as a group, is more likely that single cels to possess all the properties needed to establish metastasis e.g. cells with stem cell-like features which can adapt to growth in a new microenvironment

ii. **The site at which metastases form depends on**:

- location and vascular drainage of the primary tumour e.g. colon carcinomas often metastasize to the liver (the first capillary bed they encounter);
- tropism of certain tumour cells for specific tissues e.g. prostate carcinomas preferentially spread to bone. This may be related to tumour cells expressing certain adhesion molecules whose ligands are found preferentially on endothelial cells of the target organ, chemokine receptors that guide tumour cells to tissues expressing those chemokines, or that certain tissues provide a favourable 'soil' for tumour seedlings

iii. Once arrested at distant sites, tumour cells transmigrate between endothelial cells and through the basement membrane, which involves adhesion molecules (e.g. integrins), proteolytic enzymes and chemokines. Tumour cells then need to **escape from tumour dormancy** to grow, which likely involves secretion of cytokines, growth factors and ECM molecules that act on the resident stromal cells to make the microenvironment habitable for the cancer cell e.g. breast cancer cells metastatic to bone secrete PTHRP, stimulating osteoblasts to make RANKL which in turn activates osteoclasts to degrade the bone matrix and release growth factors supporting the growth and survival of cancer cells

8. **Ability to evade the host immune response**: tumours need to avoid elimination by the host innate and adaptive immune system, which can recognize abnormal antigens on tumour cells (immune surveillance). Therapies that neutralize these immune-evading mechanisms can therefore lead to tumour regression even in advanced cancers

- **Tumour antigens**: malignant tumours express various types of molecules that may be recognized by the immune system as "foreign"; in particular, those that elicit CD8+ cytotoxic T cell responses are most important for protective anti-tumour immunity:

a) **Neoantigens produced from genes bearing passenger and driver mutations** i.e. variant proteins that have never been seen by the immune system (likely higher in tumours with high mutational burden)

b) **Overexpressed or aberrantly expressed normal cellular proteins** e.g. tyrosinase, cancer-testis antigens. These proteins are likely normally produced in such low quantities and thus are not recognized by the immune system and fails to induce tolerance, or not normally expressed on the cell surface in an antigenic form

c) **Tumour antigens produced by oncogenic viruses** i.e. viral proteins produced in tumours associated with ongoing active or latent viral infections e.g. EBV, HPV

- **Antitumour effector mechanisms**: killing of tumour cells by **cytotoxic T cells (CTLs) specific for tumour antigens is the primary anti-tumour immune mechanism**, as most tumour neoantigens or viral antigens are endogenously synthesized and presented by MHC class I molecules, enabling their recognition by CTLs
  - CTL responses are initiated by recognition of tumour antigens on host antigen presenting cells (APCs): Dendritic cells and macrophages in the tumour microenvironment ingest necrotic/apoptotic tumour cells or released tumour antigens, and migrate to draining lymph nodes where they present the antigens in the context of MHC class II molecules as well as MHC class I molecules (through cross-presentation). Antigens can therefore be recognized by naïve CD8+ CTLs, while antigen-specific CTLs will also be activated through the expression of costimulatory molecules upregulated on APCs by "danger signals" released from damaged or necrotic tumour cells
  - Activated tumour-specific CTLs then migrate from lymph nodes to the tumour and can kill tumour cells independent of other cell types and factors

  Other mechanisms may have lesser roles in tumour immunity: antitumour CD4+ T cell responses have been detected, and in experimental systems, NK cells which require no prior sensitization and activated macrophages have also been found to be capable of killing tumour cells
- **Mechanisms of immune evasion by cancers**: Cancers can evade immune recognition or resist immune effector mechanisms even in immunocompetent hosts via several mechanisms:
  a) **Selective outgrowth of antigen-negative variants**: during tumour progression, tumour cells that survive are mostly likely those that have lost their antigens as strongly immunogenic antigen-expressing subclones may be eliminated
  b) **Loss or reduced expression of MHC molecules**: tumour cells may downregulate expression of HLA class I molecules to escape attack by cytotoxic T cells, although if still they express ligands for NK cell activating receptors, they can still trigger NK cells
  c) **Engagement of pathways that inhibit T-cell activation**: tumour cells may actively inhibit tumour immunity by upregulating negative regulatory checkpoints that normally suppress immune responses:
     - e.g. by promoting the expression of the inhibitory receptor CTLA-4 on tumour-specific T cells. CTLA-4 normally binds to and removes its ligands (B7 molecules) from APCs, thereby reducing the engagement of the activating costimulatory receptor CD28. This thus prevents sensitization and may also induce long-lived responsiveness in tumour-specific T cells
     - e.g. by upregulating PD-L1 and PD-L2 expression on tumour cells. These are cell surface proteins that activate the programmed death-1 (PD-1) receptor on effector T cells, thereby inhibiting T-cell activation
     - Immunotherapies that block CTLA-4, PD-L1 or PD-1 receptors are now approved for treatment of advanced staged cancers and certain lymphomas (aka 'checkpoint inhibitor therapies'). From these clinical trials, only a subset of tumours respond to checkpoint inhibitors e.g. tumours with high PD-L1

expression, or high tumour neoantigen burden (e.g. tumours with deficiencies in mismatch repair enzymes that normally correct DNA replication errors have high mutation burdens and are most likely to respond to these therapies). The most common associated toxicities are autoimmunity and/or inflammatory damage to a wide range of organs

d) **Secretion of immunosuppressive factors**: e.g. TGF-β is secreted In large quantities by many tumours and is a potent immunosuppressant
e) **Induction of regulatory T cells (Tregs)**: Tregs are thought to contribute to immunoevasion

Genomic instability: Intact DNA repair capabilities and other mechanisms e.g. death of cells with irreparable damage, oncogene-induced senescence and immune surveillance usually prevent cancer transformation despite exposure to mutagenic environmental agents. Genetic alterations that increase mutation rates **facilitate the acquisition of driver mutations** required for transformation and subsequent tumour progression. Sources of genomic instability include:

- **Loss of p53 function** (*see above section 'insensitivity to growth-inhibitory signals'*)
- **DNA mismatch repair factors**: acts as a "spellcheck" function to identify and correct erroneous pairings of DNA nucleotides. Without this, errors accumulate in the genome which may by chance create driver mutations. One of the hallmarks of mismatch repair defects is **microsatellite instability**. Microsatellites are tandem repeats of 1-6 nucleotides found throughout the genome, which are usually of constant length. However, if mismatch repair is defective, these microsatellites become unstable and increase or decrease in length, creating mutated alleles. Defective mismatch repair may be inherited (Lynch syndrome, an autosomal dominant disorder associated with colon carcinomas, whereby one abnormal copy of a mismatch repair gene is inherited) or acquired (due to somatic loss-of-function mutations or epigenetic silencing; the latter is more common in sporadic cancers)
- **Nucleotide excision repair factors**: repairs DNA damage (cross-linking of pyrimidine residues that prevent DNA replication) caused by UV radiation. Inherited loss-of-function mutations in any of the genes involved in nucleotide excision repair causes xeroderma pigmentosum (characterized by extremely high risk of skin cancers)
- **Homologous recombination repair factors**: repairs covalent DNA cross-links and double-stranded DNA breaks. Defects in homologous recombination factors can cause various disorders with increased risk of cancer e.g. Bloom syndrome, Ataxia telangiectasia (*ATM*), Fanconi anaemia, familial breast cancer (*BRCA1* and *BRCA2*). Defects in homologous recombination repair pathway result in activation of the salvage nonhomologous end-joining pathway, formation of dicentric chromosomes, bridge-fusion-breakage cycles and aneuploidy. *BRCA1* mutations increase risk of breast cancer, epithelial ovarian cancers and prostate cancer. *BRCA2* mutations increase risk of breast cancer in men and women, cancers of ovary, prostate, pancreas, bile duct, stomach, melanocytes and B lymphocytes
- **DNA polymerase**: normally have a very low rate or error due to an inherent exonuclease activity that allows DNA polymerase to pause, excise mismatched bases and insert the proper nucleotide

before proceeding down the template strand. Mutations in DNA polymerase can cause loss of this "proofreading" function and accumulation of numerous point substitutions, as seen in certain endometrial cancer subsets. Presumably because of the high mutation burden and neoantigens, these cancers respond well to immune checkpoint inhibitors

- **Regulated genomic instability in lymphoid cells**: Lymphoid cells normally undergo V(D)J segment recombination to assemble a variety of functional antigen receptor genes, and B cells further undergo immunoglobulin gene class switch recombination and somatic hypermutation. These regulated processes can be affected and cause lymphoid neoplasms

Cancer-promoting inflammation: It is proposed that inflammatory cells can enable many of the hallmarks of cancer either through direct interactions with tumour cells or indirectly through resident stromal cells especially cancer-associated fibroblasts and endothelial cells:

- **Release of factors that promote proliferation** e.g. EGF, or proteases that can liberate growth factors from the ECM
- **Removal of growth suppressors** e.g. proteases that degrade cell-cell or cell-ECM adhesion
- **Enhanced resistance to cell death** e.g. by expressing adhesion molecules (integrins) that promote direct physical interactions with tumour cells and preventing cell death due to detachment of epithelial cells from basement membranes and other cells (anoikis)
- **Inducing angiogenesis** e.g. through release of VEGF
- **Activating invasion and metastasis** e.g. proteases from macrophages remodel ECM, while TNF and EGF may directly stimulate cell motility
- **Evading immune destruction** e.g. TGF-β or other factors that favour the recruitment of immunosuppressive Tregs or suppress the function of CD8+ cytotoxic T cells, or expression of PD-L1 on macrophages

Mechanisms of dysregulation of cancer-associated genes

Gene mutations: Changes in a **single gene**, usually point mutations (substitution of a single nucleotide), or insertions and deletions that result in frameshift mutations

Chromosomal changes: specific recurrent changes in the **number or structure of chromosomes** are highly associated with certain tumours (as these changes usually lead to dysregulation of genes central to their pathogenesis e.g. particular oncogenes or tumour suppressor genes), and thus have diagnostic, prognostic or therapeutic implications

- **Chromosomal translocations**: most common mechanism in activating proto-oncogenes, by:
  a) **promoter or enhancement substitution** (i.e. the translocation swaps the *regulatory* elements of a proto-oncogene with another genes that is highly expressed, resulting in overexpression of the proto-oncogene). The classic example is the t(8;14) translocation in Burkitt lymphoma affecting the *MYC* gene on chr8q24 and *IGH* gene on chr14q32. In most cases, this translocation removes the regulatory sequences of the tightly regulated *MYC* gene and replaces them with the control

regions of the *IGH* gene which is highly expressed in B cells – the MYC protein is therefore constitutively expressed at high levels in Burkitt lymphoma

b) **formation of a fusion gene** (i.e. the *coding* sequences of two genes are fused, resulting in expression of a novel chimeric protein with oncogenic properties). The classic example is the Philadelphia chromosome in chronic myeloid leukemia (CML) and a subset of B-cell acute lymphoblastic lymphomas, involving chromosome breaks within the *ABL* gene on chr 9 and the *BCR* gene on chr 22. Non-homologous end-joining results in a reciprocal t(9;22) translocation that creates the oncogenic *BCR-ABL* fusion gene on the derivative chr 22 (aka Philadelphia chromosome) that encodes chimeric BCR-ABL proteins with constitutive tyrosine kinase activity

- **Chromosomal deletions**: common structural abnormality, often resulting in loss of tumour suppressor genes e.g. deletions in chr13q14, the site of the *RB* gene, are associated with retinoblastoma, and deletion of *VHL* tumour suppressor gene on chr3p is common in renal cell carcinomas. A small proportion of deletions may also activate oncogenes instead of leading to loss of gene function e.g. by juxtaposing a proto-oncogene with a nearby active promoter ("addition by genomic subtraction")
- **Amplifications**: amplified oncogenes result in their overexpression, and may be identified microscopically in two mutually exclusive patterns: (1) double minutes (multiple small extrachromosomal structures) or (2) homogeneous staining regions (HSRs) (insertion of amplified genes in new chromosomal locations lack the normal light- and dark-staining band pattern and appear homogeneous in karyotypes). Classic examples include *NMYC* amplification in neuroblastoma (associated with poor prognosis), and *ERBB2* amplification in breast cancers (which responds to antibody therapy against the HER2 receptor encoded by *ERBB2*)
- **Other changes and complex chromosomal rearrangements**: Genomic sequencing has revealed subcytogenetic rearrangements e.g. small deletions, insertions, duplications and inversions, as well larger events e.g. chromothrypsis ("chromosomal shattering") which appears to result from a single event in which dozens to hundred chromosome breaks occur in a single chromosome or several chromosomes. The pieces are reassembled haphazardly by DNA repair mechanism, resulting in many simultaneous rearrangements, deletions and even amplifications, hence expediting the process of carcinogenesis. This is particularly common in osteosarcomas and gliomas

Epigenetic changes: these refer to factors other than DNA sequence that regulate gene expression, the control of differentiation and self-renewal, and drug sensitivity and drug resistance. Epigenetics is likely the basis for why certain oncogenes and tumour suppressor genes are lineage specific, and can explain why at its extremes, the same gene can act as a tumour suppressor in one lineage and an oncogene in another (e.g. NOTCH1 gene). Because the epigenetic state of a cell depends on reversible modifications that are carried out by enzymes (which are generally good drug targets), the epigenome is a potential therapeutic target. However, as cancers likely exhibit considerable epigenetic heterogeneity from cell to cell, drug resistance can develop. Epigenetic alterations include:

- **Local DNA hypermethylation causing silencing of tumour suppressor genes**: selective hypermethylation of the promoters of tumour suppressor genes results in their transcriptional silencing. Hypermethylation typically occurs only on one allele, with the function of the tumour

suppressor gene on the other allele lost through another mechanism e.g. point mutation or deletion. E.g. hypermethylation of *CDKN2A* in several cancers

- **Global changes in DNA methylation**: abnormal patterns of DNA methylation throughout the genome may be due to mutations in genes encoding DNA methyltransferases, and can potentially result in altered (over- or under-) expression of multiple genes
- **Histone modifications**: Posttranslational modifications (histone marks) regulate gene transcription; mutations in proteins that affect histone marks or position nucleosomes on DNA or less commonly, driver mutations that affect histones themselves therefore alter gene expression

Noncoding RNAs: participate in carcinogenesis by regulating the expression of protein-coding cancer-associated genes, best characterized by microRNAs (small noncoding single-stranded RNAs that mediate sequence-specific inhibition of mRNAs). miRNAs may have tumour suppressive or oncogenic activity depending on how they regulate the expression of tumour suppressor genes or oncogenes

IV. CARCINOGENIC AGENTS

As mentioned, carcinogenesis is a multistep process. **Initiation** results from exposure of cells to a sufficient dose of a carcinogenic agent, which causes permanent DNA damage (mutations). **Promoters** subsequently lead to proliferation and clonal expansion of the initiated (mutated) cells to form a tumour mass but are not tumorigenic by themselves. Subsequent additional mutations result in the emergence of a clone with all the hallmarks of cancer

Chemical carcinogenesis: All initiating chemical carcinogens are highly reactive electrophiles that can react with nucleophilic (electron-rich) atoms in the cells, usually DNA, RNA and proteins. Interactions can cause cell death; those that cause nonlethal DNA damage are repaired in an error-prone fashion resulting in initiation of a mutated cell that passes the damage on to its daughter cells. Initiating chemical carcinogens can be:

1. **Direct-acting carcinogens**: do not require metabolic conversion to become carcinogenic. Usually weak carcinogens; includes some cancer chemotherapeutic drugs (e.g. alkylating agents)
2. **Indirect-acting carcinogens**: require metabolic conversion to become active carcinogens (ultimate carcinogens). Accounts for most chemical carcinogens, including polycyclic hydrocarbons (in fossil fuels or smoked meats and fish, or benzo[*a*]pyrene formed during the high-temperature combustion of tobacco in cigarettes and implicated in causation of lung cancer), aromatic amines and azo dyes (widely used in the past in the aniline dye and rubber industries) and aflatoxin B1 (produced by Aspergillus fungi on improperly stored grains and nuts, and associated with hepatocellular carcinoma). Most procarcinogens are metabolized by cytochrome P-450-dependent monooxygenases, the activity and inducibility of which varies significantly among individuals. Metabolic pathways are also involved in the inactivation / detoxification of certain procarcinogens or the derivatives. As such, susceptibility to carcinogenesis is partly related to the individual's polymorphic variant of these enzymes and pathways

Most chemical initiating agents target DNA and cause mutations, presumably throughout the genome. There is usually no particular predilection for certain genes or specific genetic alteration; however, because of their chemical structures, some carcinogens do interact preferentially with particular DNA sequences or bases, producing mutations clustered at 'hotspots' or enriched for particular base substitutions ('mutational signatures')

Radiation carcinogenesis: Radiant energy in the form of UV rays or ionizing electromagnetic and particulate radiation, is mutagenic and carcinogenic. Radiation may also have additive or synergistic effects with other potentially carcinogenic factors

Ultraviolet rays: UV rays from the sun is a risk factor for skin cancers (squamous cell carcinoma, basal cell carcinoma, melanoma). The degree of risk depends on the (1) type of UV rays (UVB is believed to be responsible, while UVC is filtered out by the ozone layer despite being a potent mutagen), (2) the intensity of exposure and (3) skin pigmentation (quantity of light-absorbing melanin in the skin). UVB causes pyrimidine dimers to form in DNA, particularly cross-linking of adjacent thymidine residues. This distorts the DNA helix and also prevents proper pairing with bases in the opposite DNA strand. Pyrimidine dimers are usually repaired by the nucleotide excision repair pathway; however, when this pathway is overwhelmed, error-prone nontemplated DNA repair mechanism take over that allow the cell to survive but also introduce mutations that can lead to cancer

Ionizing radiation: Electromagnetic (x-rays, γ-rays) and particulate (α particles, β particles, protons, neutrons) radiations are risk factors for several cancers, including solid tumours (lung, thyroid etc.), leukaemias. Exposure to radiation during imaging procedures e.g. CT scans has a very small but measurable increase in risk in cancers in children

Microbial carcinogenesis: Infection triggers cell proliferation, which is initially polyclonal but can become monoclonal with time due to acquisition of driver mutations in the rapidly dividing cells. Only a few viruses and rare bacterium have been linked to human cancer

Oncogenic RNA viruses

- **Human T-cell leukemia virus type 1 (HTLV-1)**: retrovirus that is the cause of adult T-cell leukemia/lymphoma (ATLL), endemic in certain parts of Japan, South America, Africa and sporadically elsewhere. HTLV-1 has tropism for CD4+ T cells, which is thus the target for neoplastic transformation. The molecular mechanism for transformation is not yet defined, but is inefficient (given the long latency period between infection and development of leukemia, and in only a small subset of infected people)

Oncogenic DNA viruses

- **Human papillomavirus (HPV)**: 70+ genetically distinct types identified
    - HPV 16 and 18 etc.: **High-risk**; implicated in squamous cell carcinomas of the cervix, anogenital region and head and neck. **Integration of viral DNA** into the host genome is important in carcinogenesis, and always occurs in a way that interrupts the viral DNA within

the E1/E2 open reading frame, leading to loss of the E2 viral repressor and increased expression of the **oncogenic E6 and E7 genes.** E6 and E7 proteins from hrHPV types has higher affinity for p53 and RB respectively compared to lrHPV
    - **E6 protein**: binds to and mediates the degradation of p53, and stimulates the expression of telomerase reverse transcriptase (TERT), the catalytic subunit of telomerase important in cell immortalization
    - **E7 protein**: binds to RB protein and displaces the E2F transcription factors, promoting cell cycle progression. Also inactivates CDK inhibitors p21 and p27, and activates cyclins A and E
  - HPV 6 and 11 etc.: **Low-risk**; associated with genital warts with low malignant potential. In benign warts, the HPV genome is maintained in a **nonintegrated episomal form**

  However, infection with HPV is not sufficient for carcinogenesis, and a high proportion of women infected with HPV clear the infection by immunologic mechanisms. HPV thus likely acts in concert with other factors e.g. cigarette smoking, co-existing microbial infections, dietary deficiencies, hormonal change and immunodeficiency
- **Epstein-Barr virus (EBV)**: ubiquitous herpesvirus implicated in various tumours including lymphomas (B-cell, NK/T-cell), nasopharyngeal carcinoma and rare sarcomas
  - **B-cell lymphomas**: EBV attaches to and infects B cells to cause latent infection (i.e. no viral replication); however the EBV proteins expressed in latently infected B cells (LMP-1, EBNA-2) allow the cells to grow indefinitely (immortalization and proliferation). In normal individuals, this EBV-driven polyclonal B-cell proliferation is readily controlled by a cytotoxic T-cell response, resulting in no symptoms or a self-limited episode of infectious mononucleosis. However, in defective T-cell immunity, these EBV-transformed B cells can produce a rapidly progressive fatal lymphoma e.g. in **B-cell lymphomas arising in immunosuppressed patients** e.g. HIV, post-transplant

    Although more than 90% of endemic **Burkitt lymphoma (BL)** tumours carry the EBV genome, out of endemic regions, EBV genome is found in only 15-20% of BL, and there are significant differences in the pattern of viral gene expression in EBV-transformed but not tumorigenic B-cell lines vs BL cells. **EBV is thus likely not directly oncogenic in BL but acts as a polyclonal B-cell mitogen** to facilitate the acquisition of the t(8;14) translocation involving the *MYC* oncogene and other mutations that ultimately produce a full-blown cancer, especially in endemic regions where cofactors such as chronic malaria impair immune competence
  - **Nasopharyngeal carcinoma (NPC)**: All NPCs (even in non-endemic regions) contain EBV. LMP-2 is expressed in NPC cells and (as in B cells) activates the NF-KB pathway to upregulate factors that may contribute to oncogenesis
- **Hepatitis B (and C) virus:** 70-85% of hepatocellular carcinomas (HCCs) are associated with HBV or HCV infections. While the oncogenic effects of HBV and HCV are multifactorial, the dominant effect appears to be **immunologically mediated chronic inflammation and hepatocyte death leading to compensatory hepatocyte proliferation during regeneration**. The activated immune cells during regeneration produce mediators such as reactive oxygen species that are genotoxic and mutagenic.

The NF-KB pathway is also activated, which blocks apoptosis and allows dividing hepatocytes to incur genotoxic stress and accumulate mutations. In addition, HBV and HCV also contains **genes that may directly promote development of cancer** e.g. *HBx* and HCV core protein (activates a variety of signal transduction pathways). HBV also integrates into the human genome, which can cause structural chromosomal changes that dysregulate oncogenes and tumour suppressor genes

- **Merkel cell polyomavirus**: cause of Merkel cell carcinoma of the skin. Viral integration into the host genome occurs, with at least 2 subsequent mutations that turn an asymptomatic viral infection into an aggressive carcinoma
- **Human herpes virus 8 (HHV8)**: cause of Kaposi sarcoma, an intermediate-grade vascular neoplasm. HHV8 causes lytic and latent infection in endothelial cells. The lytic infection causes a local inflammatory environment that favours cellular proliferation in which the latently infected cells have a growth advantage

Helicobacter pylori: first bacterium classified as a carcinogen

- H.pylori associated **gastric adenocarcinoma**: involves increased reparative epithelial cell proliferation in a background of chronic inflammation. Certain H.pylori strains also has a "pathogenicity island" that contains cytotoxin-associated A (*CagA*) gene, which can penetrate into gastric epithelial cells and stimulate growth factor pathways. The entire sequence of chronic gastritis – gastric atrophy – intestinal metaplasia – dysplasia – carcinoma takes decades to complete and occurs in only ~3% of infected patients
- **Gastric MALT (B-cell) lymphomas**: it is believed that H.pylori-reactive T cells in response to infection in turn stimulates a polyclonal B-cell proliferation, with subsequent accumulation of mutations giving rise to a monoclonal MALToma. At this stage, the MALToma remains dependent on T-cell stimulation of B-cell pathways that activates NF-KB; thus eradication of H.pylori by antibiotic therapy can be curative by removing the antigenic stimulus of T cells. However, if additional mutations are subsequently acquired that cause constitutive NF-KB activation, the MALToma will no longer be dependent on H.pylori and can spread beyond the stomach to other tissues

V. CLINICAL ASPECTS OF NEOPLASIA

Clinical manifestations: Both benign and malignant tumours can cause morbidity and mortality

- **Local effects**: Depends on tumour location; tumours can impinge on adjacent tissues and cause local pressure (mass) effects, affecting their function, causing obstruction and predispose to secondary infection e.g. a small pituitary adenoma even though benign can compress and destroy the surrounding normal gland, leading to hypopituitarism, while both benign and malignant tumours of the gut can cause intestinal obstruction, ulceration, haemorrhage
- **Hormonal effects**: Both benign and malignant neoplasm from endocrine glands can be functional (produce hormones) and cause clinical problems (although malignant tumours are more likely to be poorly differentiated and nonfunctional) e.g. a benign beta-cell adenoma of pancreatic islets can produce sufficient insulin to cause fatal hypoglycemia. Nonendocrine tumours can secrete hormones or hormone-like products (*see 'paraneoplastic syndromes' below*)

- **Cancer cachexia**: a debilitating hypercatabolic state defined by a loss of muscle mass (muscle wasting with or without loss of fat) that cannot be explained by diminished food intake. Occurs in ~50% of cancer patients and accounts for ~30% of cancer deaths (generally due to atrophy of respiratory muscles). Precise cause not known but inflammatory mediators (TNF, IL-1, IL-6) are likely important in increasing degradation of major skeletal muscle structural proteins through signaling pathways.
- **Paraneoplastic syndromes**: refers to signs and symptoms that cannot be readily explained by the anatomic distribution of the tumour or by the elaboration of hormones indigenous to the tissue from which the tumour arose. Can occur in 10% of cancer patients, and important to recognize as (1) they may be the earliest manifestation of an occult neoplasm; (2) can cause significant clinical problems or be lethal; (3) can mimic metastatic disease and affect treatment

Endocrinopathies: frequent; due to ectopic hormone production		
Cushing syndrome	*Most common endocrinopathy* • Small cell carcinoma of lung	ACTH or ACTH-like substance
Hypercalcemia	*Probably the most common paraneoplastic syndrome. Cancer-associated hypercalcemia can also be caused by osteolysis (primary in bone or metastatic to bone) but is not considered paraneoplastic* • Squamous cell carcinoma of lung • Breast carcinoma	Calcemic humoral substances e.g. parathyroid hormone-related protein (PTHRP), TGF-α, TNF, IL-1. PTHRP is produced in small amounts by many normal tissues including keratinocytes
Syndrome of inappropriate antidiuretic hormone secretion (SIADH)	• Small cell carcinoma of lung • Intracranial neoplasms	Antidiuretic hormone Atrial natriuretic hormones
Neuromyopathic: includes myasthenic syndrome, peripheral neuropathies, encephalitis etc.		
Myasthenia	• Bronchogenic carcinoma • Thymic neoplasms	Immunologic – possibly initiated by the ectopic expression of antigens normally restricted to the neuromuscular system by tumour cells. The immune system recognizes these antigens as foreign and mounts a response leading to tissue damage
Dermatologic disorders		
Acanthosis nigricans	*Grey-black thickened velvety hyperkeratotic skin patches, sometimes accompanied by abrupt development of multiple seborrheic keratosis (Leser-Trélat sign)* • Gastric carcinoma • Lung carcinoma • Uterine carcinoma	Immunologic – secretion of epidermal growth factor
Bone, joints and soft tissue changes		
Hypertrophic osteoarthropathy and clubbing of fingers	*Periosteal new bone formation primarily at the distal ends of long bones, arthritis of adjacent joints and clubbing of digits. Clubbing can also be seen in other disease e.g. chronic liver disease, diffuse lung*	Cause unknown

	disease, ulcerative colitis • Bronchogenic carcinoma • Thymic neoplasms	
Vascular and haematologic changes		
Venous thrombosis (Trousseau syndrome)	• Pancreatic carcinoma • Bronchogenic carcinoma	Tumour products (mucins) that activate clotting
Nonbacterial thrombotic endocarditis	*Bland small non-bacterial fibrinous vegetations on cardiac valve leaflets* • Advanced cancers (esp. mucin-secreting adenocarcinomas)	Hypercoagulability

Grading and staging of tumours: Systems developed to predict clinical aggressiveness of a given neoplasm for accurate prognostication and treatment. Increasingly, molecular characterization provides complementary prognostic information independent of histologic grading and clinical/pathologic staging

- **Grading**: Criteria vary in different tumour types, but generally attempt to judge the **extent to which tumour cells resemble their normal counterparts, i.e. degree of differentiation.** This is assessed under the microscope, and usually based on the degree of differentiation of the tumour cells and in some cancers, number of mitoses or architectural features. Generally range from 2-4 categories (low or high grade, or well- , moderately-, poorly-differentiated and undifferentiated). Note: histologic grade does not always correlate with biologic behaviour
- **Staging**: Judges the **extent of cancer spread;** can be assessed by **clinical** examination and imaging, or after **pathologic** examination of the resected tissue**.** Most commonly used system is the American Joint Committee on Cancer Staging (AJCC) system, which uses the TNM classification system – T (primary tumour), N (regional lymph node involvement) and M (metastases). TNM staging criteria varies in different tumour types, but generally T stage is based on increasing size or depth of the primary tumour, N stage is based on number and sites of regional lymph node involvement, and M stage is based on the presence and location of metastases. Has greater clinical implications than grade

Laboratory diagnosis of tumours: The laboratory evaluation of lesions is made in conjunction with clinical data (due to potential mimics) and depends on the quality of the tissue provided for examination

Tissue sampling methods: Sampling considerations include tissue representation (periphery vs center of the lesion) and presence of central necrosis precluding interpretation. Obtained tissue needs to be preserved in an appropriate fixative (usually formalin) before further processing for routine histology or cytology examination

- **Excision**: removal of entire lesion for evaluation
- **Incision / core biopsy**: removal of only part of lesion
- **Fine needle aspiration**: needle used to aspirate cells and fluid with subsequent cytologic examination. Less invasive and more rapidly performed than needle biopsies

- **Cytologic smears**: method of evaluation for fluids e.g. urine, pleural effusions, bronchial washing. Cancer cells with lower cohesiveness can be shed into fluids and evaluated based on their cytologic features (presence of anaplasia). Tissue invasion cannot be assessed

Frozen section: Quick (within minutes) interpretation of fresh-frozen tissue during intraoperative consultation e.g. for evaluation of surgical margins, or influencing decisions regarding extent of surgery. Can be accurate but still subject to false positive/negatives due to frozen artefacts

Ancillary techniques: used to further refine diagnosis, especially in poorly differentiated tumours or tumours difficult to distinguish based on morphologic appearance alone, prognosticate and predict response to therapy

- **Immunohistochemistry**: Specific antibodies are used to identify cell products or surface markers, useful in certain situations:
    - **Determining lineage of poorly differentiated malignant tumours** e.g. identification of cytokeratins in epithelial cell origin, desmin in muscle cell origin
    - **Identifying site of origin of metastatic tumours** e.g. tissue-specific or organ-specific antigens e.g. thyroglobulin for tumours from thyroid gland
    - **Detecting molecules with prognostic or therapeutic significance** e.g. ER, PR and cerbB2 in breast carcinomas
- **Flow cytometry**: rapidly and quantitatively measures several cell characteristics simultaneously using specific antibodies linked to different fluorescent dyes. Requires viable cells in suspension (vs immunohistochemistry, which can be performed on formalin-fixed paraffin embedded tissue), therefore usually used for characterization of haematolymphoid neoplasms
- **Molecular diagnostics and cytogenetics**: various techniques developed with different utilities
    - **Diagnosis**: not the primary modality, but helpful as ancillary technique e.g. PCR to detect presence of clonality in T or B cell neoplasms, detection of specific translocations associated with tumours especially sarcomas e.g. t(11;22) translocation in Ewing sarcoma, a small round blue cell tumour
    - **Prognosis**: certain genetic alterations are associated with poor prognosis e.g *NMYC* gene amplification in neuroblastoma
    - **Detection of minimal residual disease**: e.g. through PCR-based amplification of nucleic acid sequences unique to the malignant clone in acute leukemia
    - **Diagnosis of hereditary predisposition to cancer**: detection of germline mutations which can then allow closer follow-up, possible prophylactic surgery and genetic counselling of relatives who may also be at-risk e.g. *BRCA1* and *BRCA2*
    - **Guiding targeted therapy**: increasing number of therapeutic agents are targeted to oncoproteins present only in a subset of cancers e.g. crizotinib for lung cancers with ALK gene rearrangements

Molecular profiling and '-omics': recent years have seen the development of technologies that can rapidly sequence an entire genome (genomics), assess epigenetic modifications genome-wide (epigenomics), quantify all the RNAs expressed in a cell population (transcriptomics), measure many

proteins simultaneously (proteomics) and create a snapshot of all of a cell's metabolites (metabolomics). The systematic sequencing and cataloguing of genomic alterations in various human cancers is largely stored within The Cancer Genome Atlas (TCGA) sponsored by the National Cancer Institute. This has facilitated research with subsequent clinical translation: 'targeted' sequencing is now routinely performed in many large cancer centers with reasonable costs and turnaround times to identify therapeutically "actionable" genetic lesions e.g. in lung cancers which are genetically diverse and require a "personalized" approach for targeted therapy to succeed. Such molecular profiling techniques will complement morphologic histopathologic evaluation for diagnosis, inform prognosis and guide treatment

Tumour markers: refers to tumour-associated enzymes, hormones and other substances in the blood detected via biochemical assays. Although they lack the sensitivity and specificity for diagnosis, they can contribute to the detection of cancer in conjunction with other modalities, and can monitor a tumour's response to therapy and recurrence e.g. prostate-specific antigen (PSA) for prostatic adenocarcinoma, levels of which can also be elevated in benign conditions like benign prostatic hyperplasia, alpha-fetoprotein (AFP) in hepatocellular carcinoma, yolk sac tumours

Circulating tumour cells (CTCs), cell-free DNA and RNA: still mainly in clinical research phase rather than routine clinical care. Instruments that can detect, quantify and characterize rare solid tumour cells in blood or cell-free DNA shed from dying tumour cells into the blood ('**liquid biopsies'**) can potentially permit earlier diagnosis, gauge risk of metastasis, provide a minimally invasive method of assessing response to therapy and identify mechanisms of drug resistance