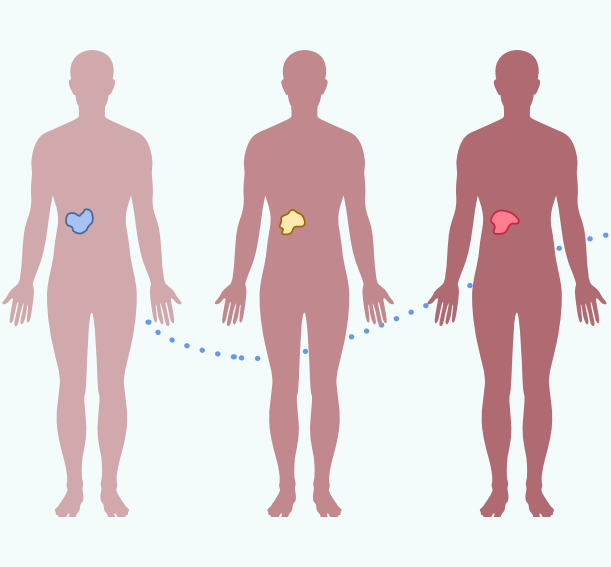
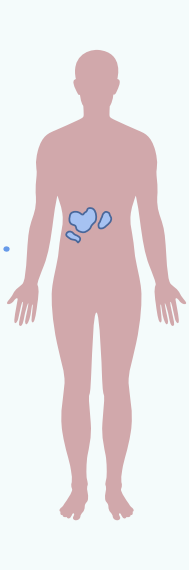
Technology Networks

TUMOR HETEROGENEITY

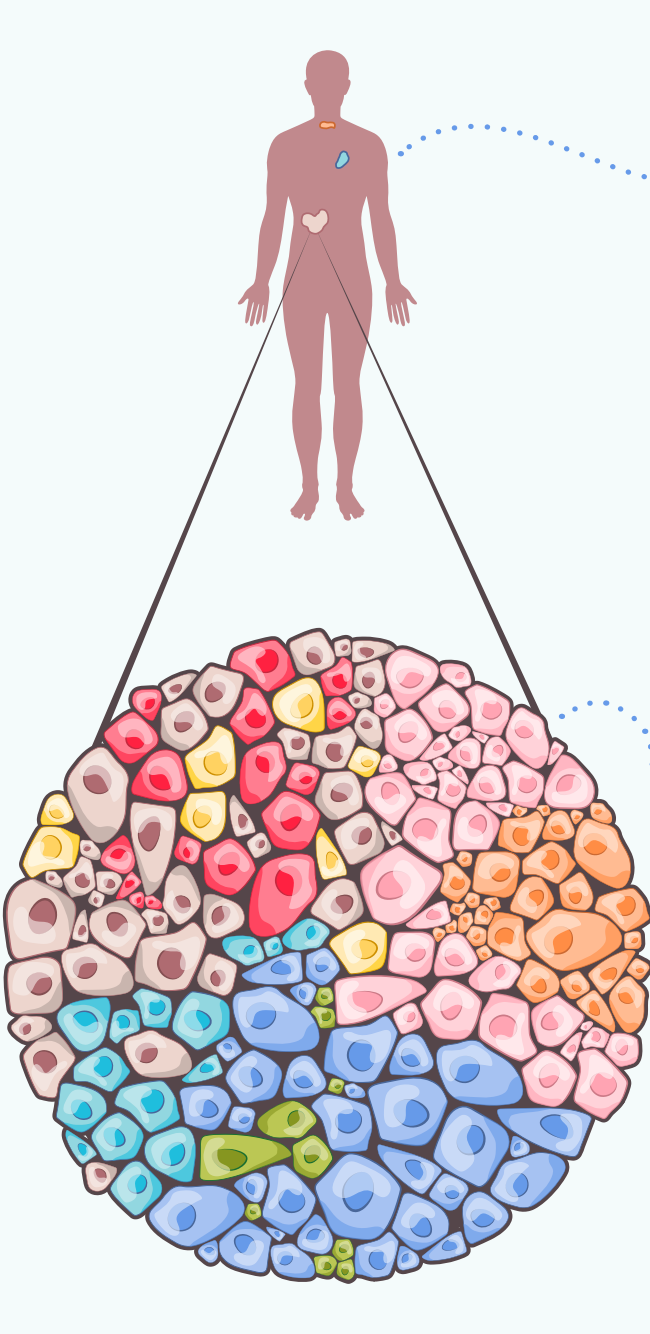
Cancer is an extremely complex group of diseases. As it progresses, tumors can evolve to comprise varied cell types with distinct morphological and genetic features. This diversity – or heterogeneity – is reflected at many levels, not only between patients, but within tumors from a *single* patient. Here we take a closer look at the various levels of heterogeneity.



INTERPATIENT HETEROGENEITY describes the variations among tumors from different individuals.[1]



MULTIFOCAL DISEASE occurs when multiple tumors arise from a unique cellular clone in a particular organ in a single patient.[2]



INTRAPATIENT INTERTUMOR HETEROGENEITY describes the variations between an individual patient's primary tumor and metastatic tumor, as well as variations among numerous metastases.[1]

INTRATUMOR HETEROGENEITY occurs when different subpopulations of cancer cells, with distinct genetic and phenotypic characteristics, are located within a single tumor in an individual patient.[3]

INTRATUMOR HETEROGENEITY IN FOCUS

Intratumor heterogeneity is driven by various mechanisms, which can be broadly grouped as:[4]

INTRINSIC FACTORS
Properties of a cell that contribute to its disease phenotype

EXTRINSIC FACTORS
Components of a cell's microenvironment that influence its disease phenotype

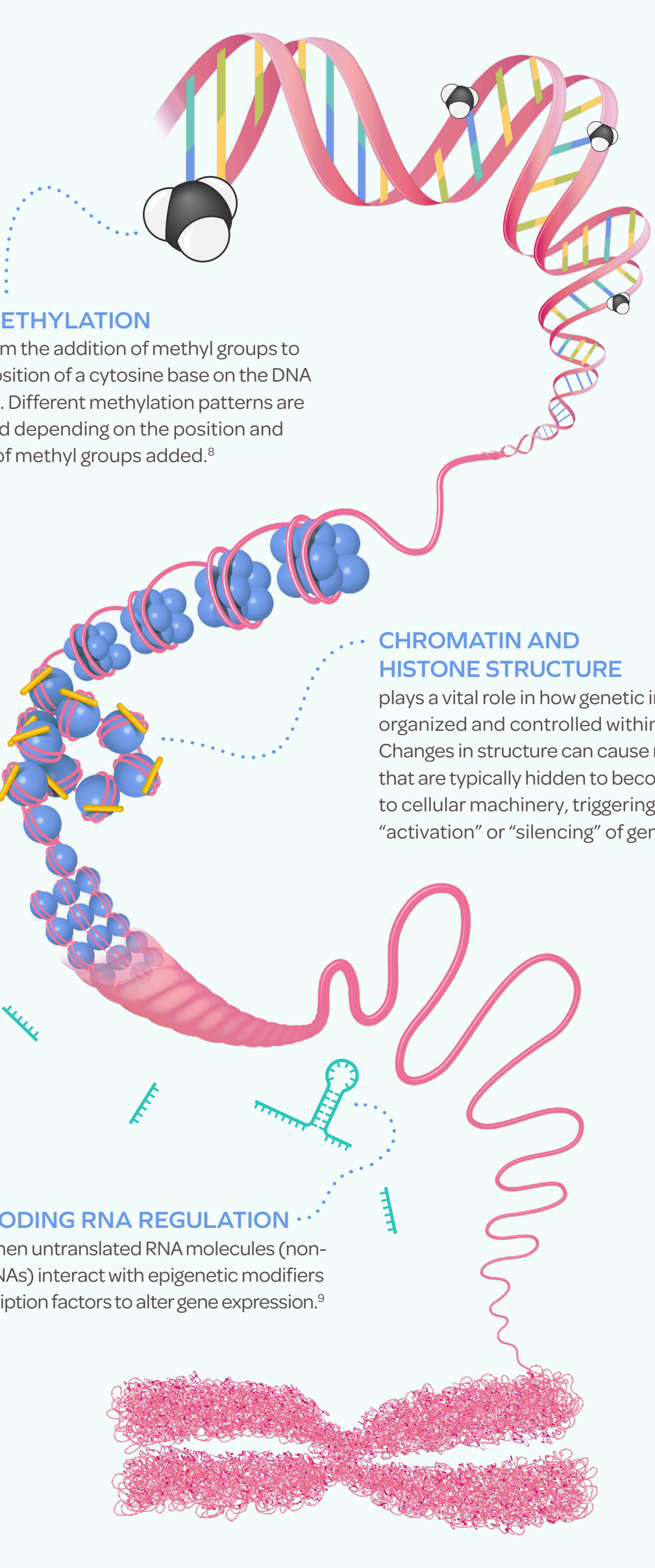
INTRINSIC FACTORS

Intrinsic triggers of heterogeneity can be **epigenetic**, **genomic** or **transcriptomic/ proteomic** in origin.[1,3]

1 Epigenetic heterogeneity

Cell populations that are indistinguishable genetically can be morphologically diverse. This is due to aberrant epigenetic modifications, of which there are different kinds. By studying the epigenome of cancer cells, it is possible to:[3,5,6,7]

- Track the evolution of subpopulations within the tumor
- Determine a tumor's potential to develop therapy resistance
- Identify potential epigenetic therapies that may be able to reverse or delay resistance.



DNA METHYLATION arises from the addition of methyl groups to the C5 position of a cytosine base on the DNA molecule. Different methylation patterns are generated depending on the position and number of methyl groups added.[8]

CHROMATIN AND HISTONE STRUCTURE plays a vital role in how genetic information is organized and controlled within a single cell. Changes in structure can cause regions of DNA that are typically hidden to become accessible to cellular machinery, triggering aberrant "activation" or "silencing" of genes.

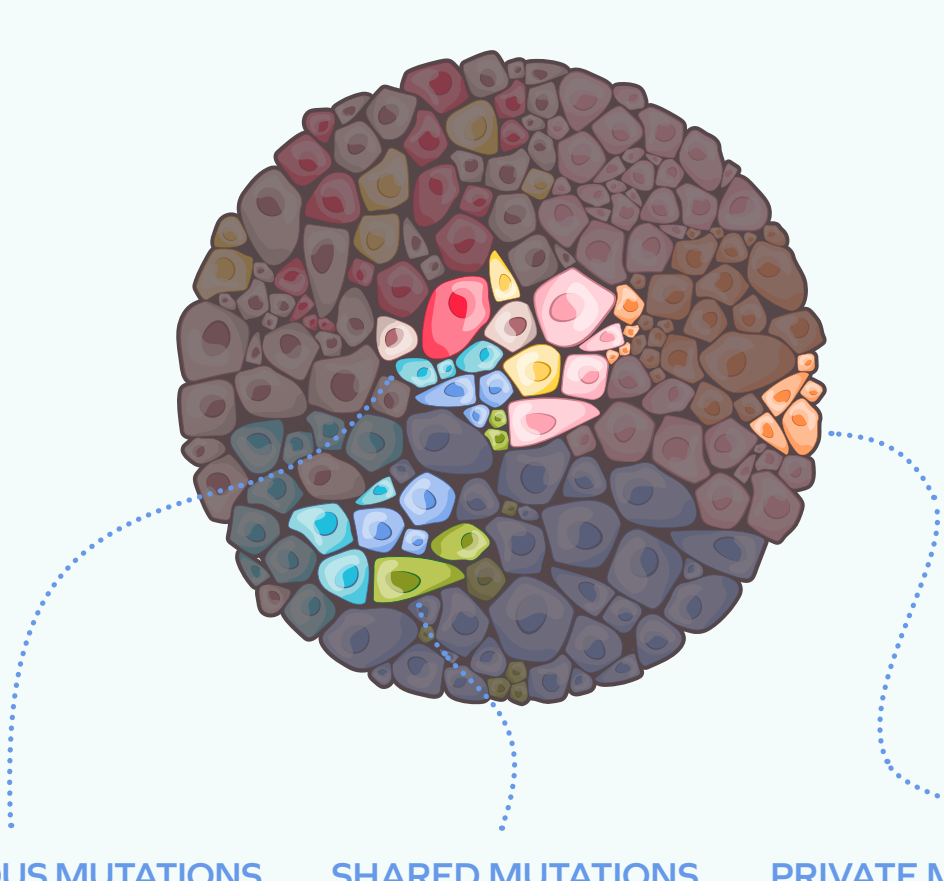
NON-CODING RNA REGULATION occurs when untranslated RNA molecules (non-coding RNAs) interact with epigenetic modifiers or transcription factors to alter gene expression.[9]

2 Genomic heterogeneity

While most genetic changes are classed as "passenger events" with no benefit to cancer cells, a few will provide a selective advantage.

Genomic instability, a hallmark of cancer, can be the direct result of an inhibited or reduced DNA damage response within a cell, or may be caused by extrinsic factors such as hypoxia acting on the cell.

There are three different types of mutation that can occur during tumor evolution[1]...



UBIQUITOUS MUTATIONS
- occur early on
- present in all cancer cells within the tumor

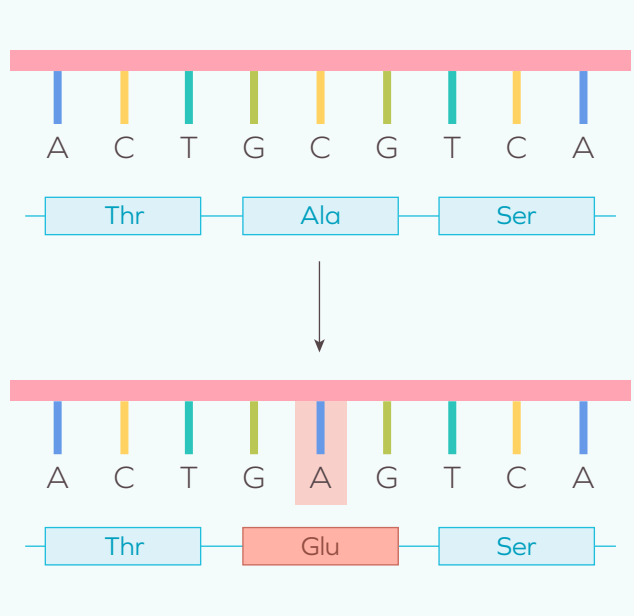
SHARED MUTATIONS
- occur during tumor progression
- present in some regions of the tumor

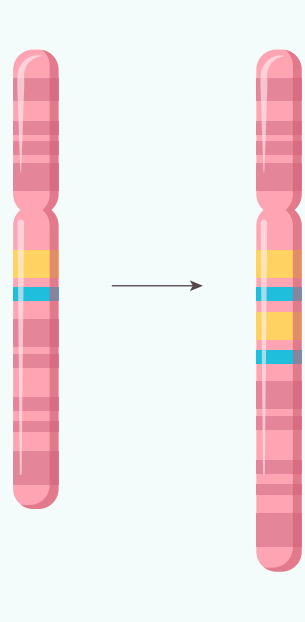
PRIVATE MUTATIONS
- occur latest in tumor progression
- present in one subpopulation of cells
- form the basis of **genomic intratumor heterogeneity**

Tumors can harbor anything from a couple of mutations to thousands, resulting in the generation of subpopulations of cells and various degrees of heterogeneity.[1,10,11]

Here we take a closer look at some of the mutations that can occur, both at the DNA sequence and chromosomal level...

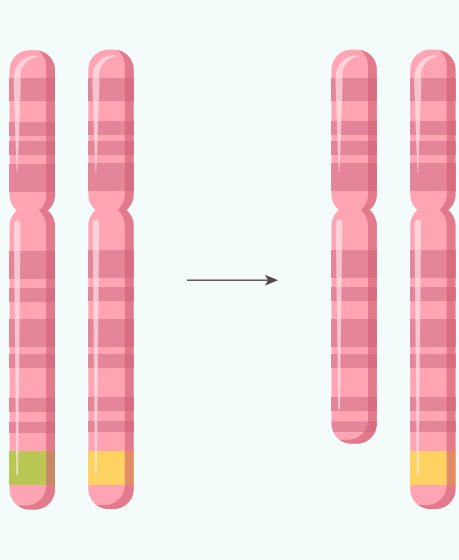
SINGLE NUCLEOTIDE VARIANTS arise when a single nucleotide – adenine, thymine, cytosine or guanine – is changed.[12]

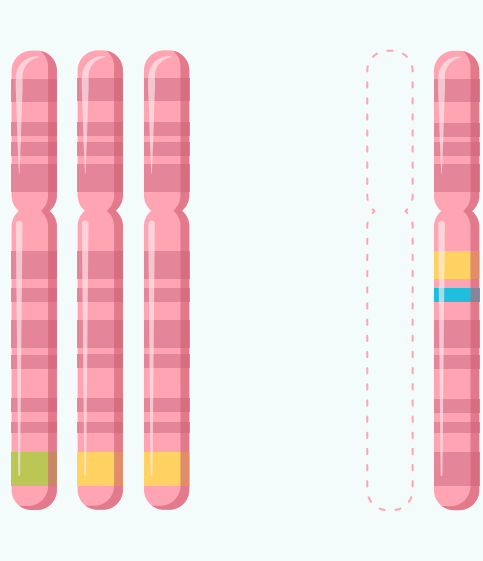




COPY NUMBER VARIATION is a structural change whereby a section of DNA is repeated. The number of copies can vary between individual cells.[13]

LOSS OF HETEROZYGOSITY is a form of allelic imbalance, whereby a cancer cell loses one of its two alleles at a particular locus.[14]





KARYOTYPE ABERRATIONS result in an abnormal number of chromosomes in some cells within the tumor (aneuploid cells). More complicated karyotypes occur as a cancer becomes more advanced.[15]

Depending on what you want to learn about the tumor will influence the choice of sequencing assay used:[10]

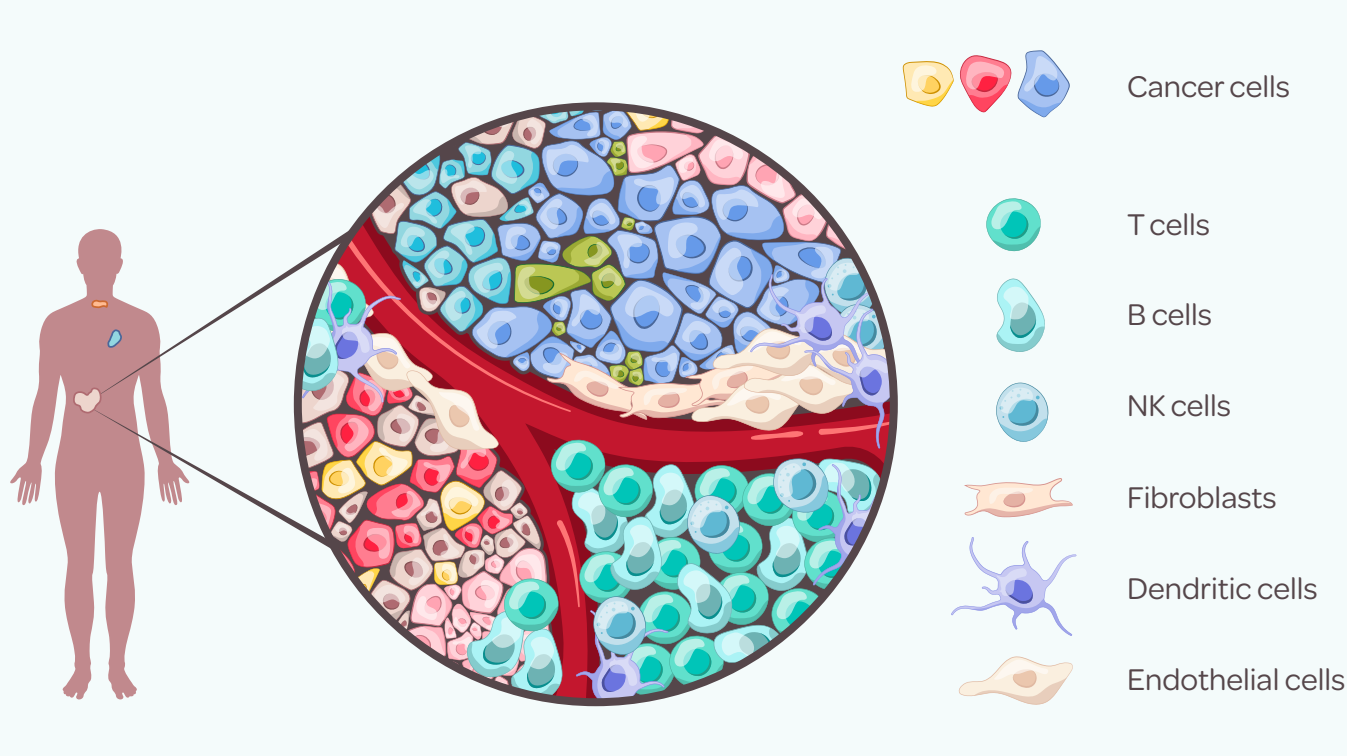
DEEP SEQUENCING
is used to determine the frequency of subpopulations of cells within a tumor

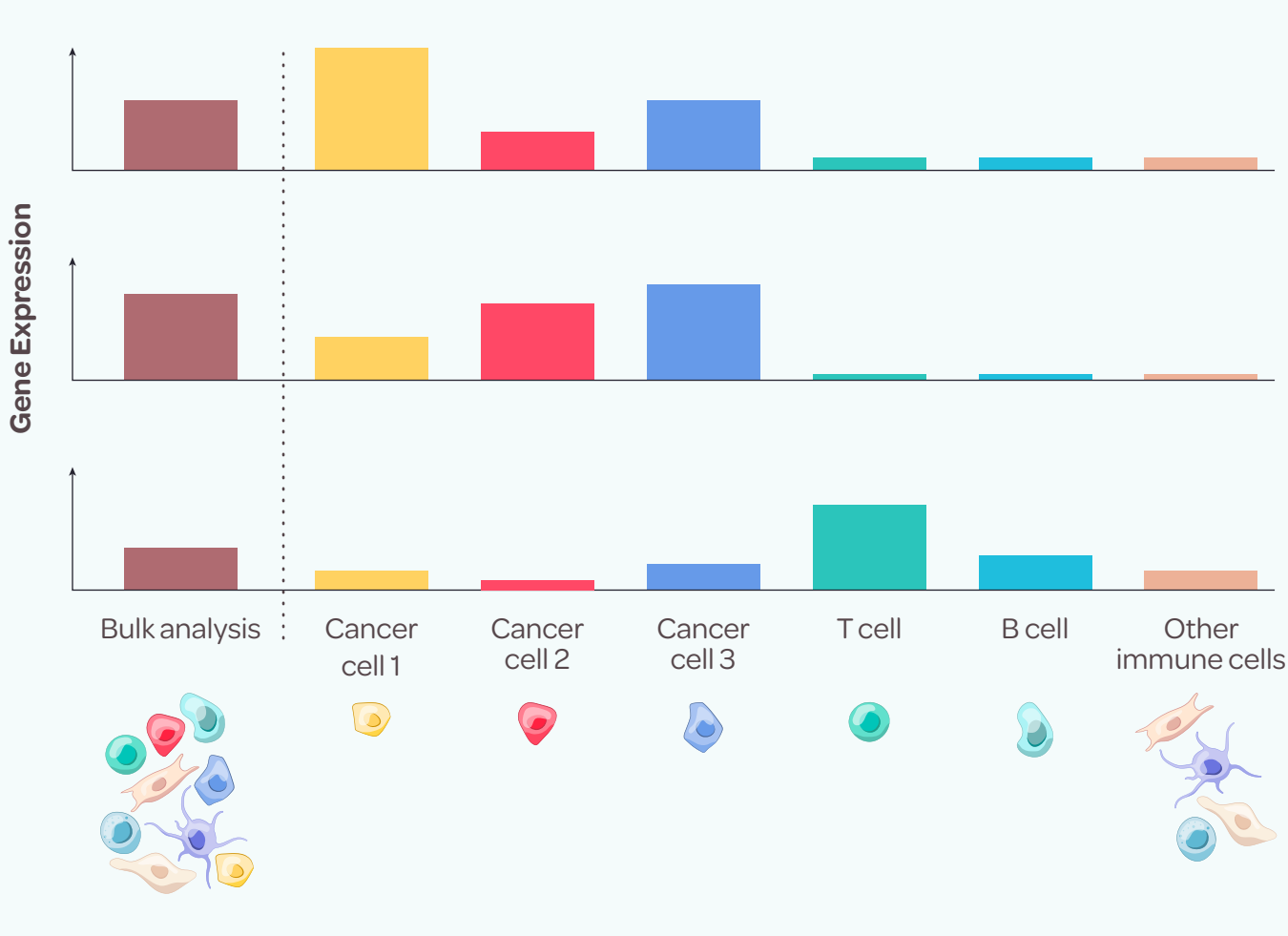
GENOME-WIDE SEQUENCING
enables the detection of passenger mutations that uniquely identify distinct subpopulations

3 Transcriptomic/ Proteomic heterogeneity

While a cell's genetic profile is fairly stable, its transcriptional profile can change as it grows, differentiates and responds to local signaling events within the tumor's microenvironment. Transcriptional intratumor heterogeneity is therefore highly dynamic and can vary based on the location of the tumor in a patient's body.

By performing transcriptomic profiling at **single-cell** resolution, using methods such as single-cell RNA sequencing, it is possible to measure the molecular mechanisms responsible for the diversity of cells within a tumor that are undetectable with bulk analysis.[16]





By studying the proteome, it is possible to gain insights into *in situ* disease biology that, when obtained from large cohorts of cancer patients, can help to identify conserved and divergent proteomic alterations (interpatient heterogeneity). Beyond this, proteomic analysis of biopsy samples, taken from various regions within a *single* tumor, can reveal intratumor heterogeneity.[17]

Histochemical and immunohistochemical techniques can be used to detect protein targets within the tumor tissue and to reveal spatial information. Pairing techniques, for example *in situ* hybridization and immunohistochemistry, can help to better characterize the spatially chaotic tumor microenvironment.[18]

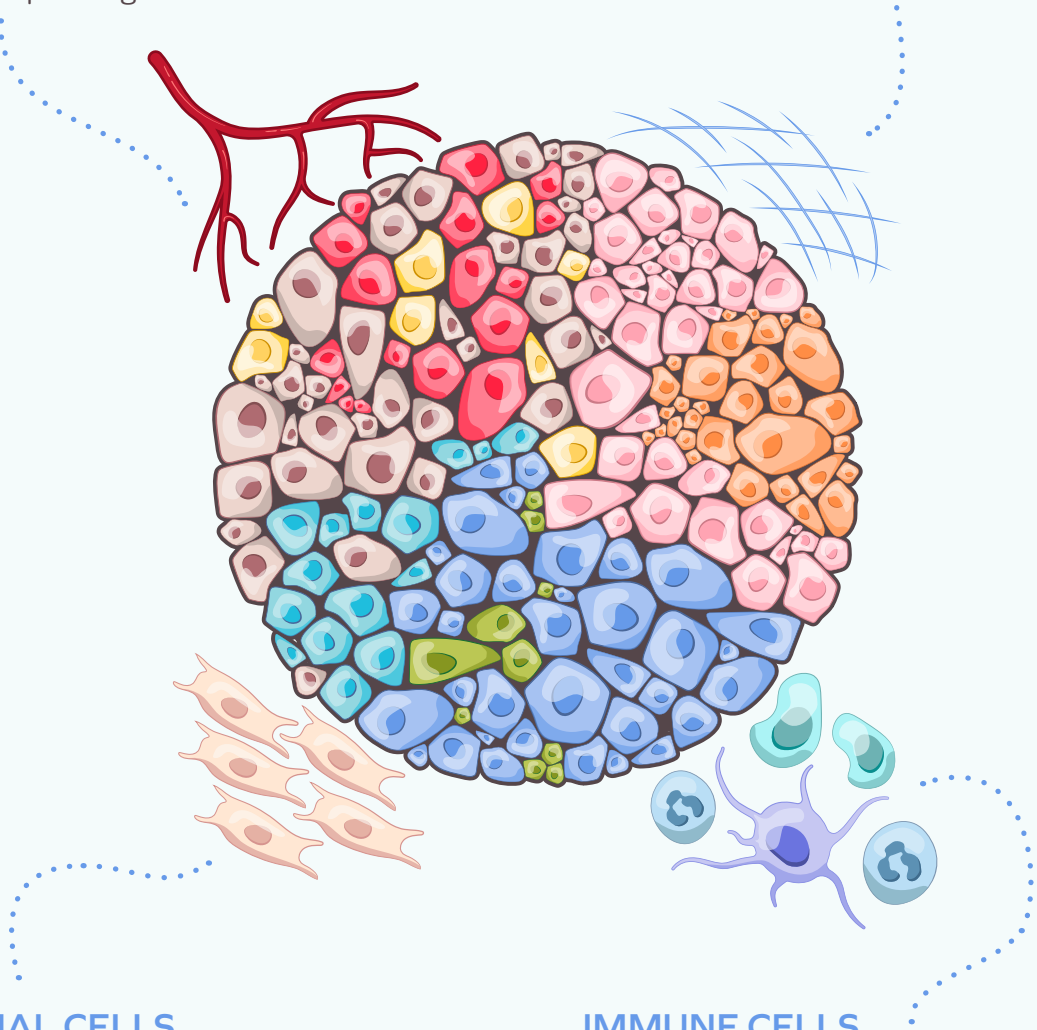
EXTRINSIC FACTORS

Extrinsic triggers of heterogeneity include components of the tumor microenvironment. The tumor microenvironment is a complex "ecosystem" comprising diseased cells, healthy cells of various types including immune cells, secreted factors and extracellular matrix proteins.[19,20]

Differences in the environment surrounding the tumor and the dynamic and bi-directional interactions between various components of the microenvironment cause genotypic and phenotypic changes to the cancer cells within.

SUPPLY OF BLOOD AND NUTRIENTS
In contrast to normal vasculature, tumor blood vessels are dilated and unevenly distributed throughout the tumor. This results in some cancer cells receiving a greater supply of oxygen and nutrients compared to others, creating different microenvironments within the tumor and varying degrees of heterogeneity as cells adapt to regional conditions.[19]

COMPOSITION OF THE EXTRACELLULAR MATRIX (ECM)
The ECM can associate with receptors on the surface of cancer cells, stimulating intracellular signaling pathways, which can alter the cells' phenotype.[22]



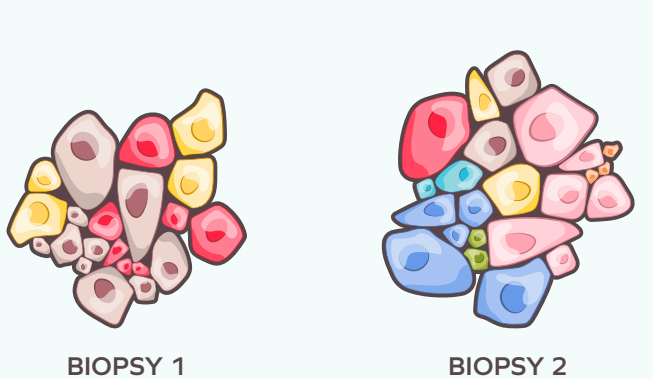
STROMAL CELLS
such as fibroblasts, can modify the ECM, causing it to release matrix bound growth factors and degradation products that stimulate the infiltration of immune cells, promote the generation if new blood vessels and act directly on cancer cells within the vicinity.[22]

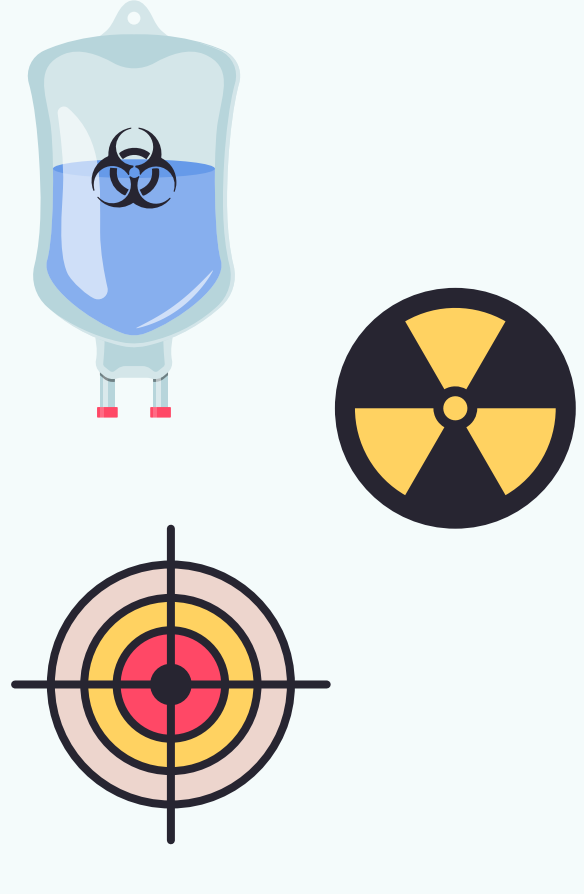
IMMUNE CELLS
comprising the innate and adaptive immune system are present within the tumor microenvironment. Pro-inflammatory cytokines released by immune cells can alter DNA methylation and non-coding RNA expression. Immune surveillance at the tumor site has also been shown to evolve as a tumor evolves. T cell clones can track neoantigens on cancer cells both spatially and temporally.[21,23]

CLINICAL IMPLICATIONS OF INTRATUMOR HETEROGENEITY

Intratumor heterogeneity can impact a patient's diagnosis, prognosis and treatment response. Traditionally a patient's clinical assessment would be based on a single tumor biopsy, however, as we now know, this approach doesn't capture a tumor's heterogeneity.

DIAGNOSIS AND PROGNOSIS[1]
- It is necessary to obtain and assess multiple biopsies, for all tumors, to ensure the full spectrum of heterogeneity is captured.
- A higher degree of heterogeneity is typically associated with a poorer prognosis, due to the present of many subpopulations of cancer cells.





TREATMENT RESPONSE AND RESISTANCE[1,3]
- **Chemotherapy** and **radiotherapy** can trigger the selection of resistant subpopulations of cancer cells, introduce new genetic mutations in the cells creating additional subpopulations and drive the development of subpopulations indirectly by influencing components of the tumor microenvironment.
- **Targeted cancer therapies** work by targeting specific changes which are unique to a particular cancer. Knowing exactly what subpopulations of cells are present within the tumor helps to determine the most effective therapy and assess the risk of resistance.

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