

Molecular Profiling of Tumors

Integrating tumor genomes, expression profiles and histopathology

From sequencing readouts to clinically meaningful interpretation

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Learning outcomes

Map molecular layers

Distinguish genome, epigenome, transcriptome, proteome and metabolome readouts.

Interpret a profile

Explain targeted sequencing, coverage, alteration classes and signatures.

Connect to care

Relate tumor profiles to target selection, therapy response and predictive assays.

Integrate morphology

Show why histology, microenvironment and molecular data remain complementary.

The central diagnostic problem

Morphology asks

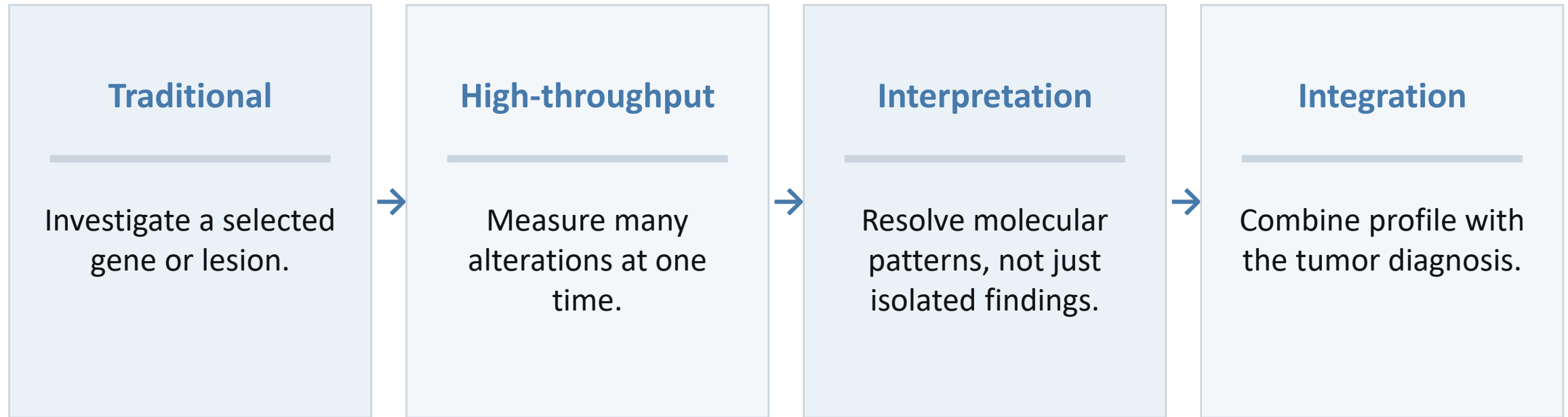
- What kind of tumor is present?
- How invasive, heterogeneous and differentiated is it?
- What is the tissue and stromal context?

Molecular profiling asks

- Which genetic or expression alterations occur?
- Are actionable targets or pathway defects detectable?
- Do molecular patterns inform treatment selection?

Neither information stream is a complete substitute for the other.

From one gene to a molecular profile



The conceptual shift is breadth of measurement and integrated interpretation.

The “omics” landscape

Genome

DNA sequence and genomic alterations.

Epigenome

Genome-wide regulatory modifications.

Transcriptome

RNAs expressed by the cell population.

Proteome

Many expressed proteins measured together.

Metabolome

A snapshot of cellular metabolites.

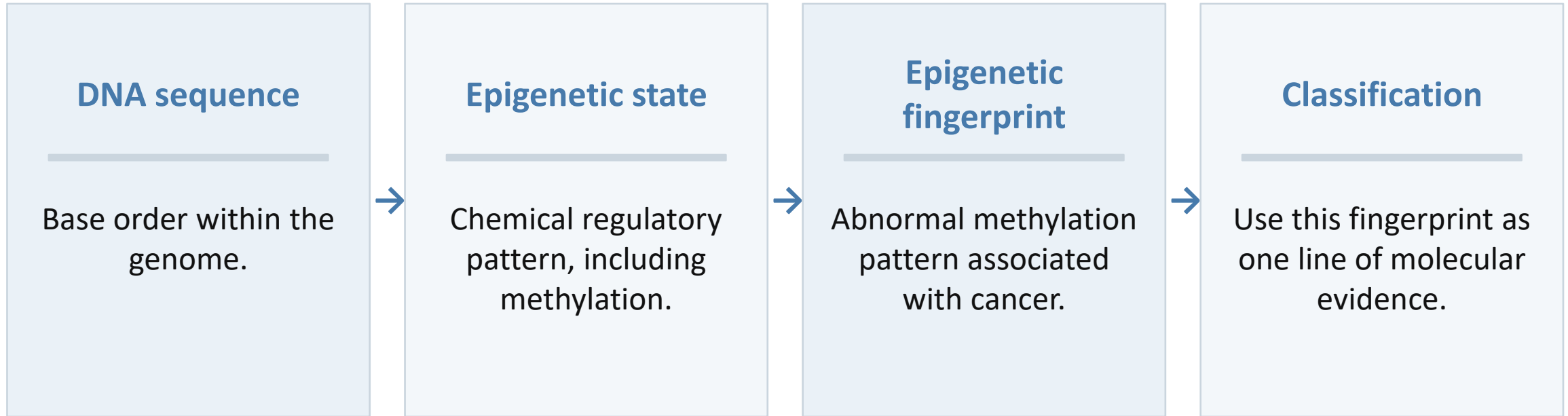
Genomics measures altered DNA

- High-throughput sequencing surveys genomic lesions across multiple genes.
- Cancer genomes harbor sequence and structural changes of different types.
- A DNA profile supplies potential diagnostic and therapeutic information.
- Genomic measurement alone does not describe cell architecture or local immunity.

Interpretation

DNA profiles
characterize alterations;
They do not replace
tissue interpretation.

The epigenome is a distinct layer



Epigenetic classification is not interchangeable with sequence mutation detection.

Transcriptomics measures expressed RNA

- RNA abundance provides a readout of expressed genes.
- RNA profiling and DNA sequencing answer different biological questions.
- RNA-based assays can identify expressed gene-fusion transcripts.
- Expression patterns can assist estimates of treatment benefit in selected tumors.

Interpretation

A DNA change identifies a lesion; An RNA assay assesses expressed transcripts.

Proteomics measures tumor proteins

Readout

Measures many proteins simultaneously.

Biological layer

Represents products of gene expression.

Interpretation

Protein-level patterns are distinct from DNA sequence changes.

Proteomics among global molecular approaches; This passage does not specify a clinical assay.

Metabolomics measures biochemical state

Readout

Collective snapshot of cellular metabolites.

Biological layer

Reflects downstream cellular chemical activity.

Interpretation

Describes a different axis than genomic and transcriptomic profiles.

No tumor-specific metabolomic cutoff or treatment test is established in the assigned passage.

Different omics, different questions

Layer	What is measured?	Illustrative question
Genomics	DNA variants	Which alterations are present?
Epigenomics	Regulatory modifications	What methylation pattern exists?
Transcriptomics	RNA abundance / transcripts	What is expressed?
Proteomics	Proteins	What proteins are present?
Metabolomics	Metabolites	What chemical state is captured?

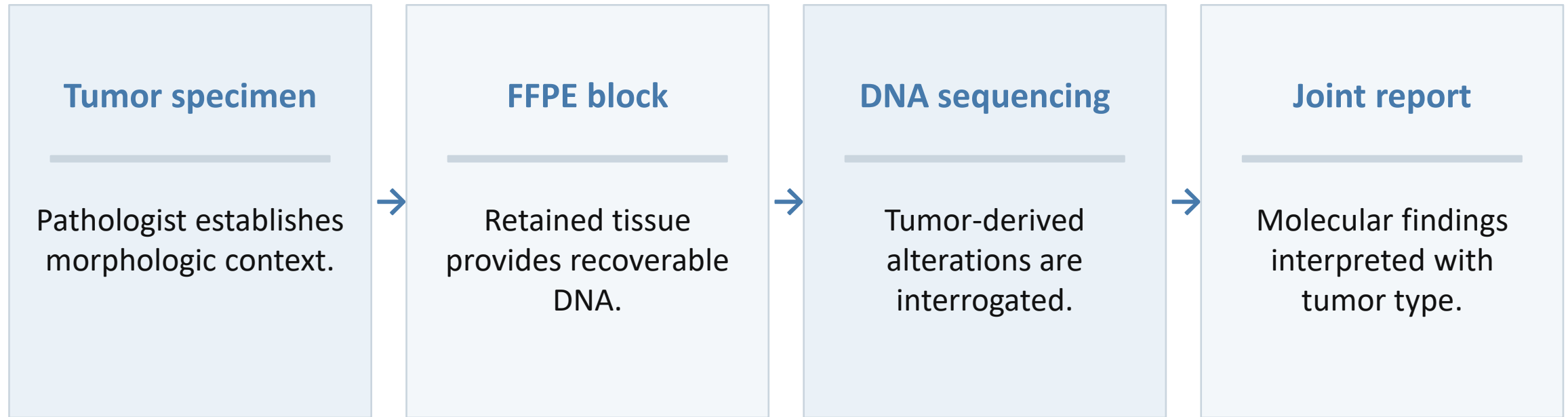
Why DNA profiling entered pathology first

- Sequencing as the most clinically influential omics platform.
- DNA can be recovered from formalin-fixed, paraffin-embedded tissue.
- Such tissue blocks are routinely retained by pathology departments.
- This specimen compatibility accelerated clinical use of high-throughput assays.

Interpretation

Routine archival tumor material made sequencing operationally practical.

FFPE tissue links histology and genomics



Schematic clinical pathway; Not an assay validation diagram.

A profile is derived from sampled tumor

The molecular sample

- Provides DNA or RNA from sampled material.
- Reflects the portion of tumor actually analyzed.
- May reveal multiple genomic alterations.

The tissue section

- Reveals invasive architecture and anaplasia.
- Shows within-tumor heterogeneity.
- Retains stromal and immune context.

Sampling and morphology shape how a profile should be understood.

TCGA: Systematic cancer genome cataloging

- The Cancer Genome Atlas (TCGA) is a major NCI-sponsored consortium.
- Large-scale studies systematically cataloged genetic alterations across cancer types.
- The resulting complexity motivated integrative genomic displays.
- Research cancer catalogs and an individual clinical report serve different purposes.

Interpretation

A genomic atlas maps patterns; A clinical report interprets a patient's tumor.

Cancer genomes contain multiple lesion classes

Sequence variants

Point mutations and other changes in nucleotide sequence.

Copy-number changes

Regions of increased or decreased genetic material.

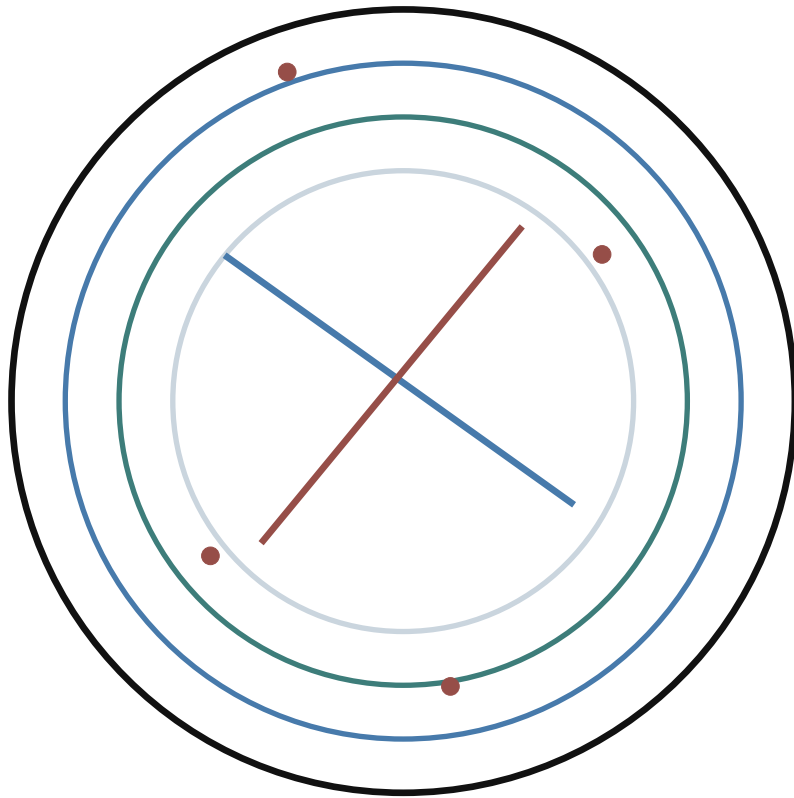
Allelic imbalance

Unequal representation of alleles, including loss of heterozygosity.

Rearrangements

Structural changes within or between chromosomes.

Circos plots: An integrative view



Schematic rings, not patient data

Chromosome ring

Genomic locations on a circular axis

Rearrangement links

Within-/between-chromosome events

Track layers

Allelic imbalance and copy-number changes

Mutation marks

Point mutation positions

Diagrammatic representation of the tracks Fig. 7.48

Structural rearrangements in profiles

Intrachromosomal

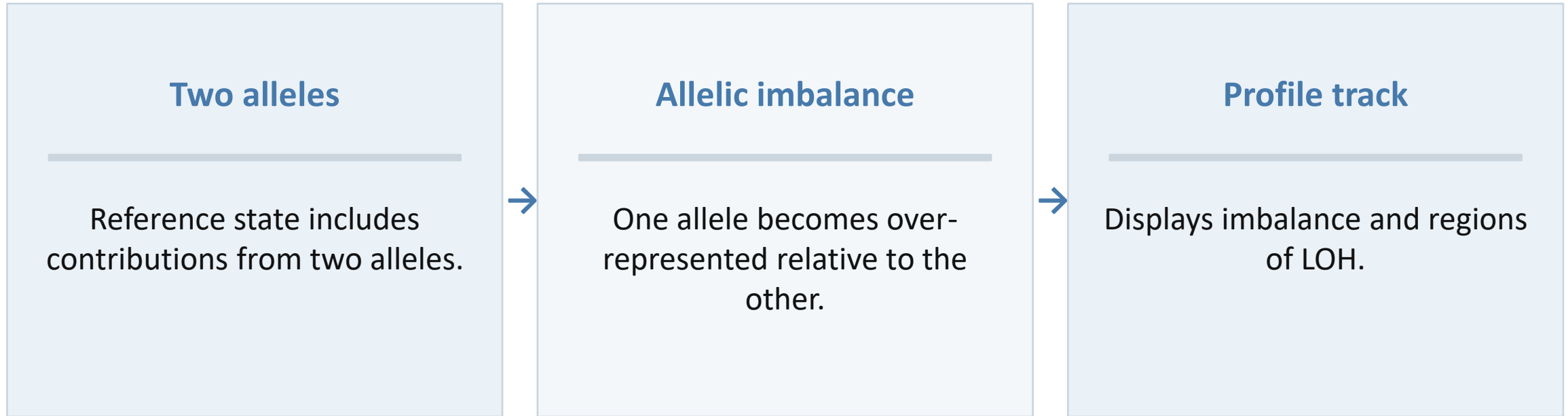
- Structural change within one chromosome.
- In the referenced circos plot, represented with blue links.

Interchromosomal

- Rearrangement involving different chromosomes.
- In the referenced plot, represented with red links.

Link location matters: These categories are not point mutations.

Loss of heterozygosity and allelic balance



The caption of Fig. 7.48 displays LOH/imbalance as a separate track.

Copy-number gain versus loss

Copy-number gain

- Increased genomic material in a region.
- Shown as gain on a copy-number track.
- May occur alongside other alteration classes.

Copy-number loss

- Reduced genomic material in a region.
- Shown as loss on a copy-number track.
- Is distinct from a sequence point mutation.

Gain and loss are relative copy-number categories, not invented counts.

Point mutations are another profile layer

- Point mutations alter the DNA nucleotide sequence at a position.
- In Fig. 7.48, point mutations are represented by dots on a genomic track.
- A point mutation may be a driver or a passenger.
- Interpretation depends on the precise alteration and tumor context.

Interpretation

The presence of a dot is not equivalent to an actionable result.

Reading a multi-layer genome view

Track / link	Encoded information	Interpretation
Chromosome ring	Genomic coordinate	Where the event lies
Structural links	Within/between chromosomes	How regions are rearranged
Allelic track	LOH / imbalance	Relationship of alleles
Copy-number track	Gain / loss	Relative regional DNA dosage
Mutation marks	Sequence alterations	Sites of point mutation

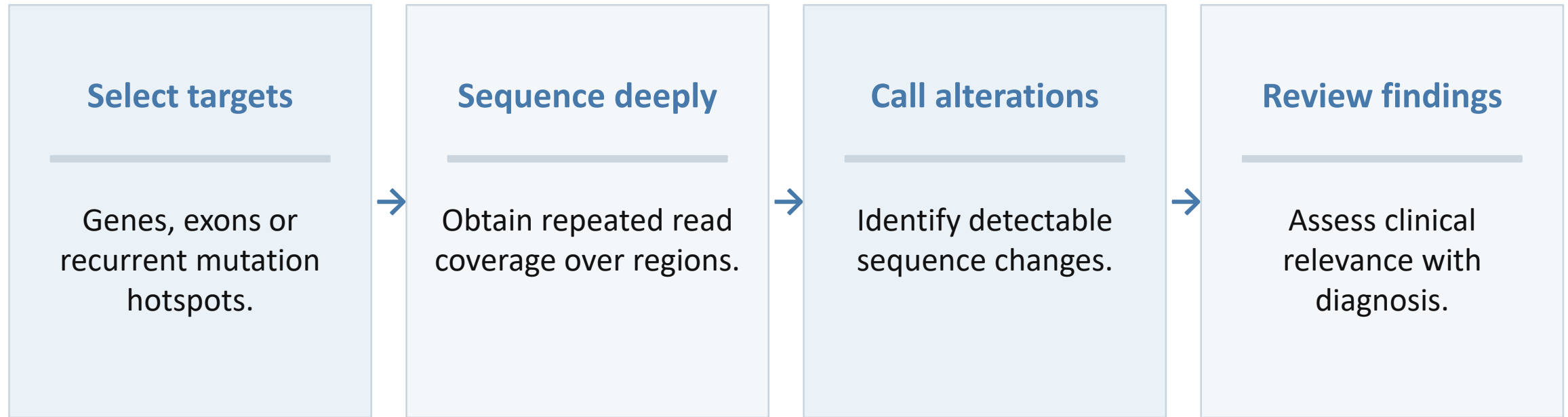
Next-generation sequencing in cancer

- NGS permits many genes or regions to be sequenced in parallel.
- The assigned text describes routine targeted testing in cancer centers and reference laboratories.
- Clinical panels apply to both solid and hematologic malignancies.
- A profile is interpreted to answer a clinical question, not merely to list variants.

Interpretation

A broad sequencing readout requires tumor-specific clinical interpretation.

Targeted sequencing narrows the search



Targeted sequencing maximizes attention on selected cancer-related regions.

Panels and hotspots are not identical

Hotspot strategy

- Emphasizes recurrently mutated positions.
- Provides focused analysis of predefined loci.
- Does not interrogate unselected regions.

Multi-gene panel

- Can cover exons from hundreds of genes.
- Expands the range of alteration opportunities.
- Still remains bounded by assay design.

The profile cannot establish absence of abnormalities outside its designed targets.

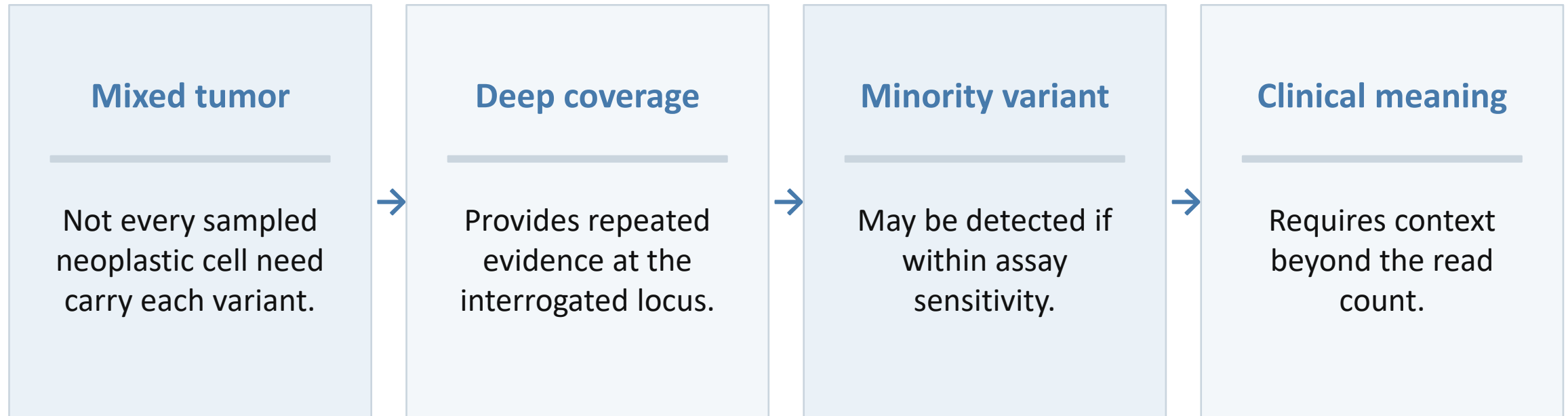
Sequencing depth is fold coverage

- Depth denotes how many sequence reads cover the region under interrogation.
- Greater depth can support detection of variants present in minority populations.
- Panels designed to detect mutations in small tumor subpopulations.
- Analytical sensitivity depends on more than the number of targets on a panel.

Interpretation

Depth describes read coverage, not the fraction of tumor in a tissue section.

Minor variants and tumor heterogeneity



Schematic illustration of why sequencing depth matters; No simulated frequencies.

What a negative panel cannot prove

Limited regions

An alteration outside the targeted region may not be assessed.

Detection limit

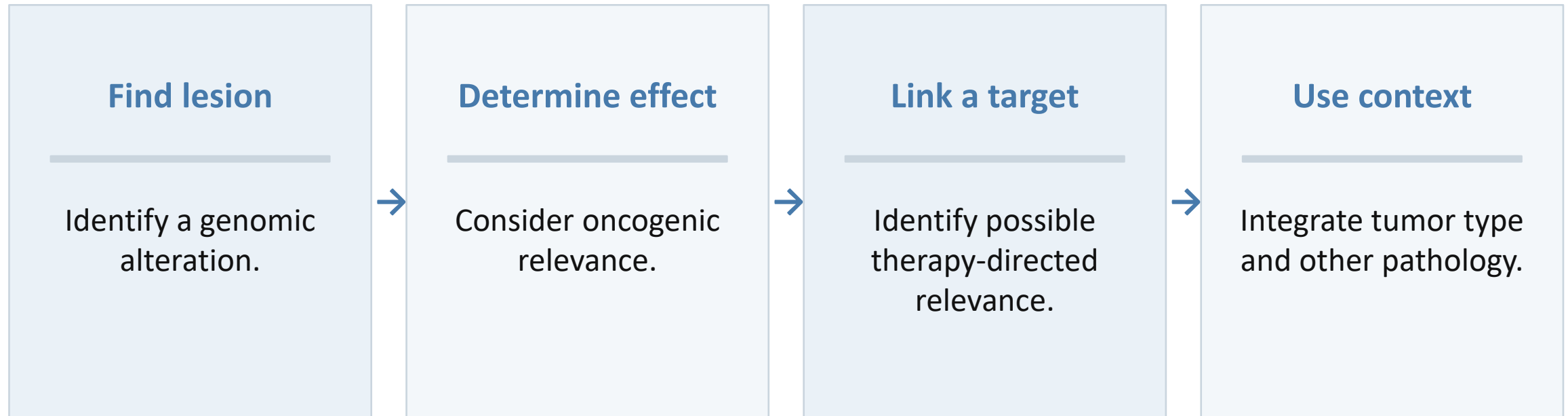
A low-abundance variant may fall below sensitivity.

Interpretation

A negative targeted panel is not a tumor-wide declaration of genetic normality.

These limitations follow from targeted coverage and the role of sequencing depth.

Actionability: The aim of many panels



A genomic lesion is not automatically a treatment indication.

A driver is not every observed mutation

Driver alteration

- Contributes to malignant cell growth or survival.
- May be therapeutically relevant in a defined tumor.

Passenger alteration

- Accompanies tumor development without acting as the principal driver.
- Can still contribute to the overall mutation pattern.

Both types can be visible in a tumor-wide sequence profile.

Lung carcinoma illustrates molecular diversity

- Lung carcinomas comprise genetically diverse neoplasms.
- A common histologic label does not imply a single targetable alteration.
- Molecular typing is especially relevant when selecting a targeted strategy.
- Tissue diagnosis remains essential for interpreting which findings matter.

Interpretation

For genetically diverse tumors, classification and treatment selection need different kinds of information.

Examples of treatment-relevant lesions

Tumor setting	Illustrative lesion	Clinical use described
Lung cancer	EGFR alteration	Guide oncoprotein-directed treatment
Lung cancer	ALK rearrangement	Identify a therapeutically relevant driver
Melanoma	BRAF mutation	Identify potential BRAF-directed treatment
CML	BCR–ABL fusion	Molecular diagnosis and treatment guidance

Clinical interpretation is not gene matching

Molecular evidence

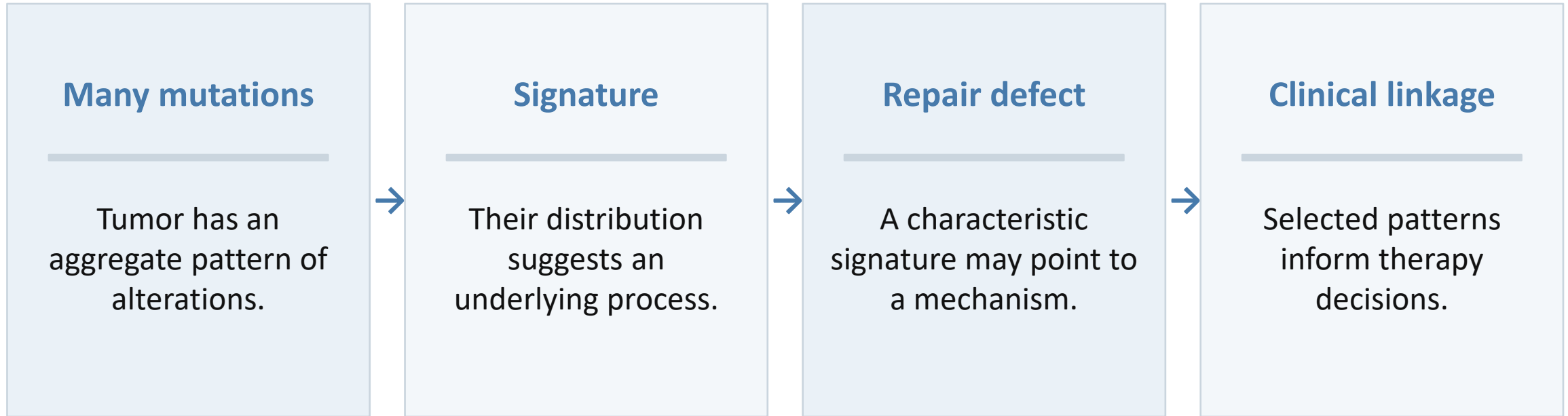
- Exact lesion is identified.
- Biologic or therapeutic mechanism is considered.
- A putative target is recognized.

Pathology evidence

- Tumor lineage and subtype are established.
- Morphology documents invasion and differentiation.
- Tissue-specific treatment behavior is relevant.

This is why a target list is not a complete cancer diagnosis.

Mutation patterns add information



Patterns may use passenger as well as driver mutations.

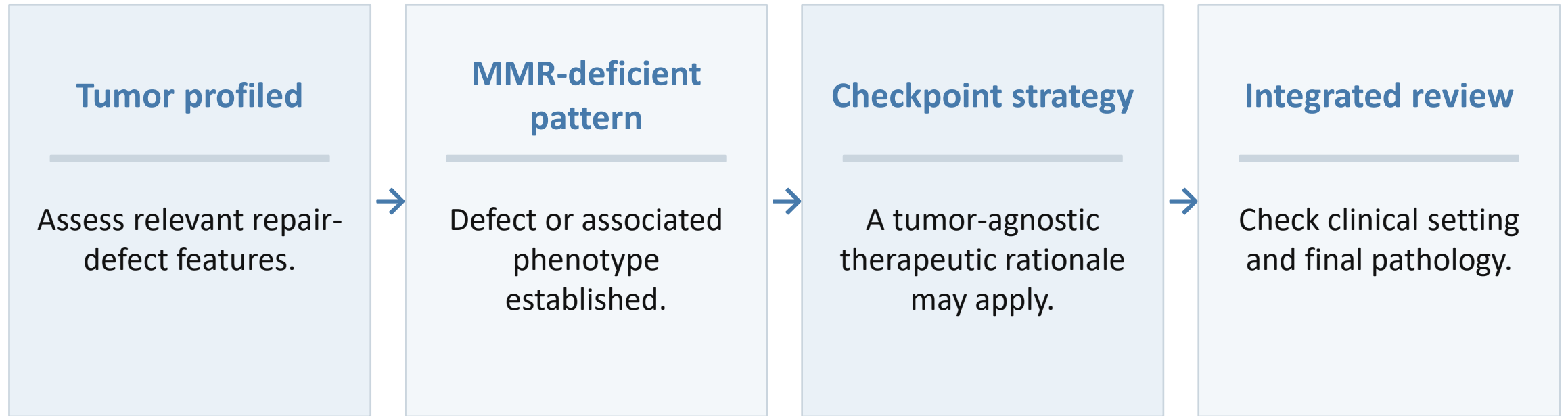
Microsatellite instability and MMR

- Mismatch repair (MMR) defects produce a characteristic abnormal mutation phenotype.
- Microsatellite instability (MSI) in this setting.
- The assay finding can carry clinical meaning beyond a single driver gene.
- MSI is a molecular phenotype; It should not be equated with any isolated mutation.

Interpretation

Interpret the defect or phenotype, not a solitary mutation name.

MMR deficiency can redirect therapy



Checkpoint blockade based on MMR defects across tumor types.

Tumor-agnostic is a qualified concept

Histology-based decision

- Start with anatomic site and tumor subtype.
- Integrate stage, morphology and lineage.

Molecularly defined decision

- A shared molecular defect may support a cross-tumor therapeutic approach.
- Example: MMR deficiency and checkpoint blockade.

A molecular indication does not erase the diagnosis or other clinical requirements.

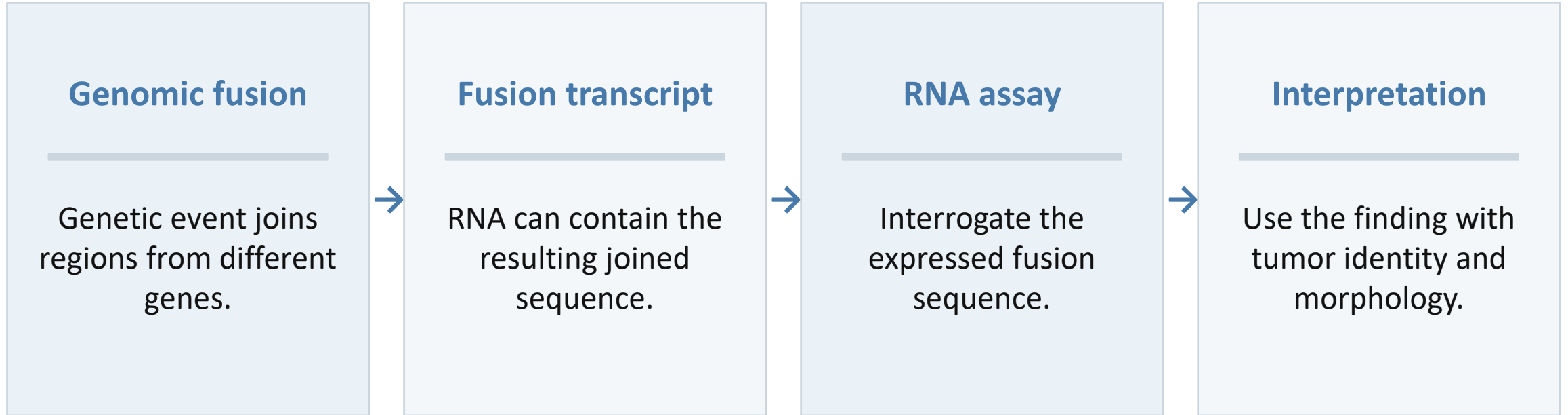
RNA presents technical challenges

- RNA is more vulnerable to degradation than DNA.
- Fixed tissue can still yield clinically useful RNA readouts.
- The RNA analyte and assay must be suited to the intended question.
- Continuing platform development to improve RNA measurement.

Interpretation

DNA and RNA workflows cannot be treated as analytically interchangeable.

Fusion transcripts are RNA targets



RNA sequencing and RNA-based assays for fusion detection.

Anchored RT-PCR adds diagnostic reach

- Anchored reverse-transcriptase PCR characterizes tumor-derived fusion transcripts.
- The method characterizes fusion transcripts from fixed tumor tissue.
- The approach broadens the ability to recognize tumor-specific RNA rearrangements.
- Fusion evidence assists classification when morphology alone is insufficient.

Interpretation

Fusion-transcript analysis can complement morphology when several tumor entities appear similar.

Breast cancer uses expression profiling

- Breast-cancer RNA-based tests are described as routine in the source.
- Expression profiling can help assess whether chemotherapy is likely to benefit a patient.
- The clinical question is treatment benefit, rather than simply tumor detection.
- This is an example of predictive use of a multi-gene molecular readout.

Interpretation

Specific assay thresholds and eligibility criteria are not supplied by this excerpt.

Diagnostic, prognostic, predictive

Purpose	Clinical question	Profile-related example
Diagnostic	What neoplasm is present?	Molecularly defined subtype
Prognostic	What clinical course is expected?	Genomic alterations linked to outcome
Predictive	Will a therapy likely help?	Breast expression profile / actionable lesion

Classification by molecule or morphology?

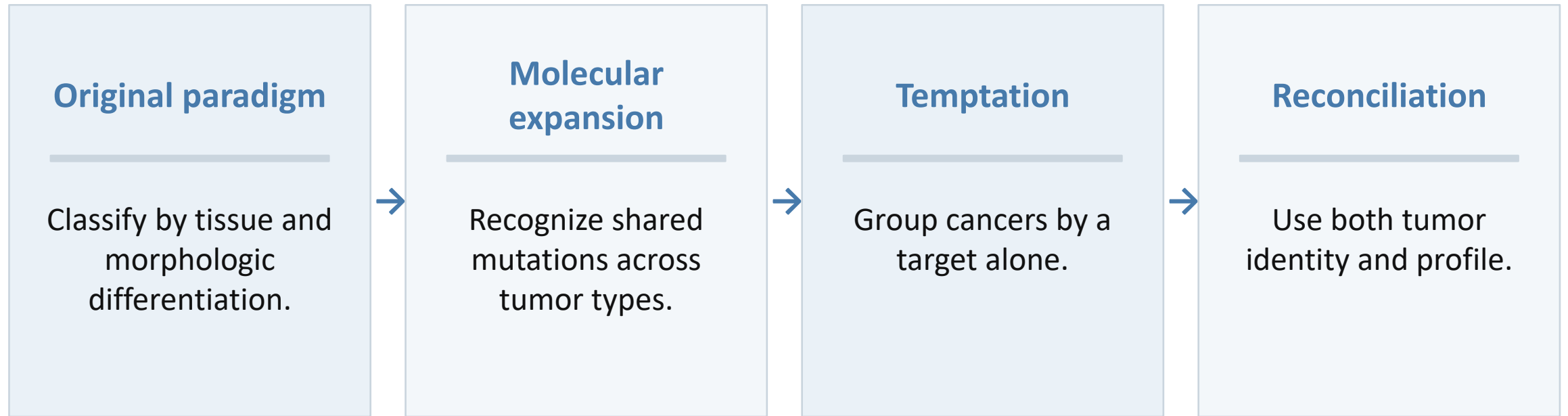


Figure 7.49 contrasts tissue-origin and target-centered approaches.

Shared mutation, distinct malignancies

Hairy cell leukemia

One BRAF-mutated tumor setting in the source.

Melanoma

A morphologically different BRAF-mutated cancer.

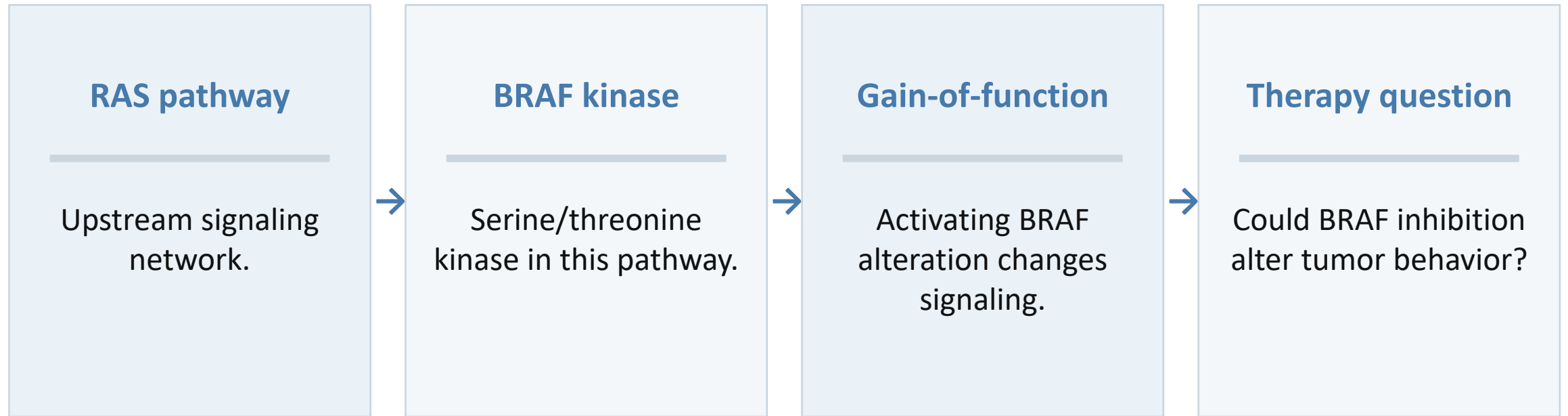
Colorectal carcinoma

BRAF lesions may also occur in colorectal carcinoma.

Other tumor examples

Papillary thyroid carcinoma and Langerhans cell histiocytosis also appear in Fig. 7.50.

BRAF links genotype to MAPK signaling



Original schematic reflects only the pathway relation stated in the source.

BRAF inhibition is context dependent

BRAF-mutated tumor	Response pattern described	Teaching implication
Hairy cell leukemia	Typically sustained response	Genotype may be highly informative
Melanoma	Often transient response	Response may not endure
Colorectal carcinoma	Limited response in source account	Genotype alone is insufficient

Qualitative examples reproduced; Not modern regimen-specific efficacy estimates.

The molecular target is not the whole tumor

- A shared BRAF alteration does not yield identical responses in every tissue.
- The source notes that the reasons for divergent inhibitor responses are uncertain.
- Histologic identity retains predictive and diagnostic context.
- Therapy selection therefore requires more than a mutation-matched drug label.

Interpretation

Molecular similarity must not be mistaken for clinical equivalence.

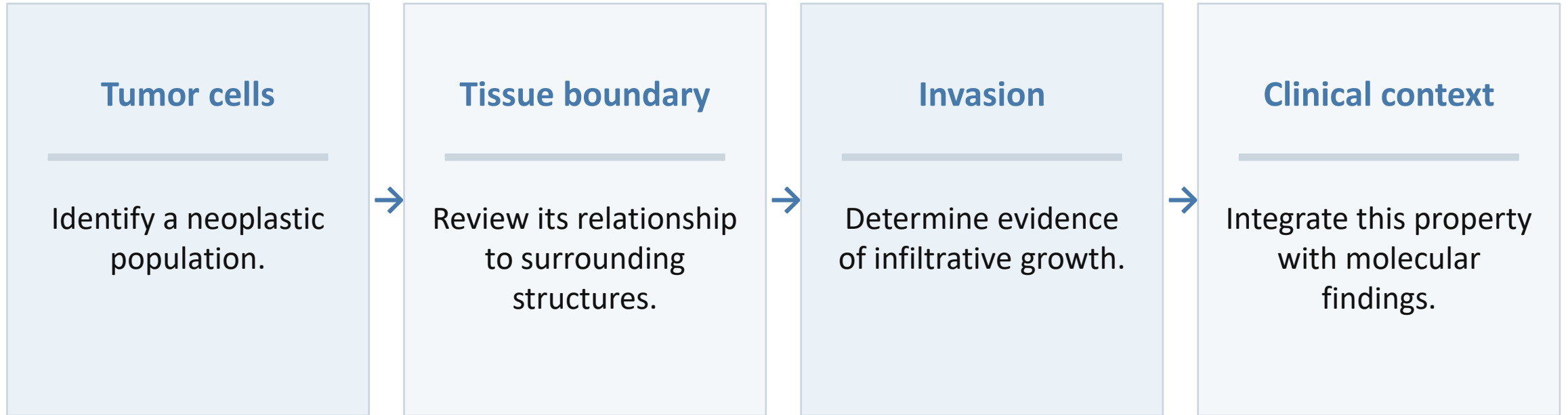
Anaplasia is visible in tissue

- Histopathology evaluates tumor differentiation and anaplasia.
- These properties are represented spatially in actual tissue architecture.
- A sequence-based gene list does not directly show the degree of anaplasia.
- Combined reports preserve both phenotypic and genomic information.

Interpretation

Morphologic interpretation contributes information a DNA readout does not encode directly.

Invasion is a morphologic relationship



This is a conceptual analytic workflow, not a depiction of a histology specimen.

Tumor heterogeneity spans multiple scales

Molecular

Different alterations may be present within tumor populations.

Histologic

Morphology may vary across regions of the same tumor.

Microenvironmental

Stroma, vessels and immune cells differ by region.

Spatial context explains why a bulk profile and a section view are complementary.

Stroma and angiogenesis matter

- Tumors interact with surrounding non-neoplastic stromal elements.
- Angiogenesis is one tissue-level property visible in the tumor environment.
- Molecular profiling alone does not display vascular architecture.
- Pathology interpretation links neoplastic cells with the supporting tissue.

Interpretation

The tumor is a tissue system, not merely a list of somatic variants.

Host immunity is part of the phenotype

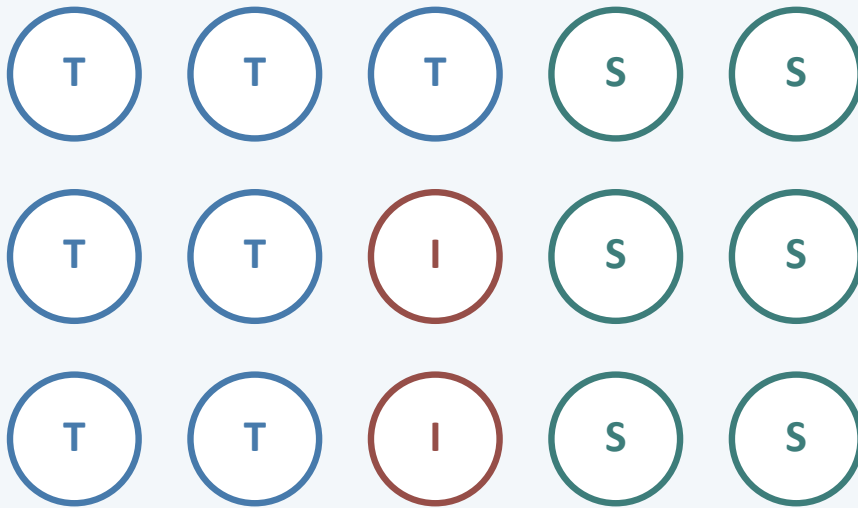
- Host immune responses can be evaluated in relation to the tumor.
- Immune evasion has therapeutic relevance.
- Global tumor molecular data and tissue immunity interrogate different domains.
- Spatially resolved tools aim to connect expression with cell and tissue location.

Interpretation

The immediate immune context may influence interpretation of therapies.

Spatial transcriptomics adds location

Illustrative tissue map



T: Tumor S: Stromal I: Immune

Conventional bulk RNA

Expression across the sampled population

Spatial RNA profiling

Expression associated with tissue position

Integrated reading

Relate tumor, stroma and immune-cell locations

Schematic only — no observed cells or expression measurements

Bulk and spatial RNA readouts differ

Bulk transcriptome

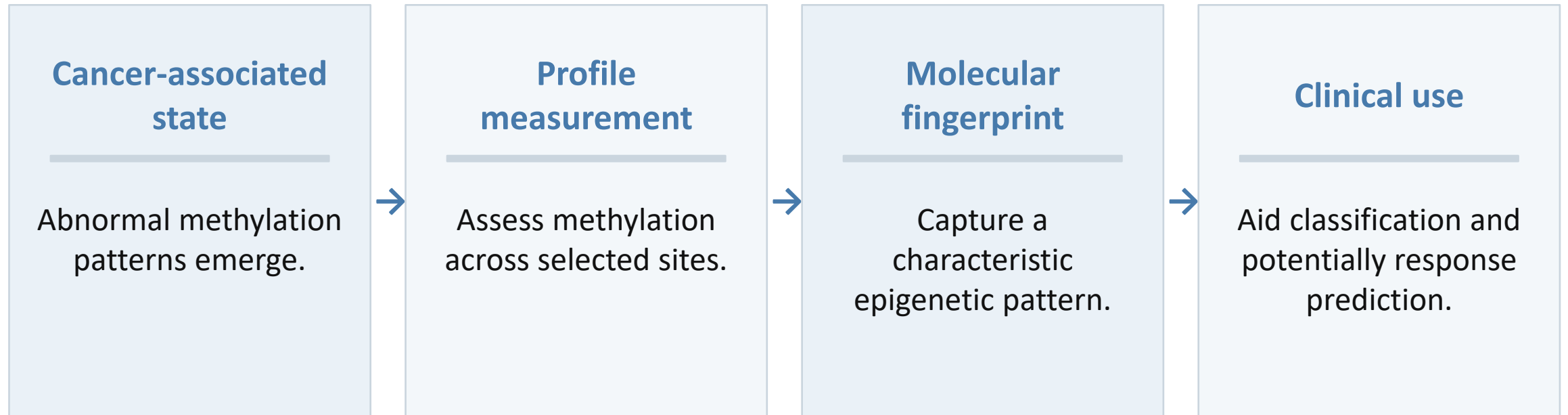
- Aggregates RNA from a sampled population.
- Characterizes overall expression patterns.
- Cannot independently recover full tissue topology.

Spatial transcriptomics

- Couples expression to mapped tissue locations.
- Can explore tumor–stroma and immune relationships.
- Still requires morphologic interpretation.

These approaches differ chiefly in whether spatial position is retained.

DNA methylation is a tumor fingerprint



Methylation changes are epigenetic, not alterations of the DNA base sequence.

Methylation profiling has a different role

DNA sequencing

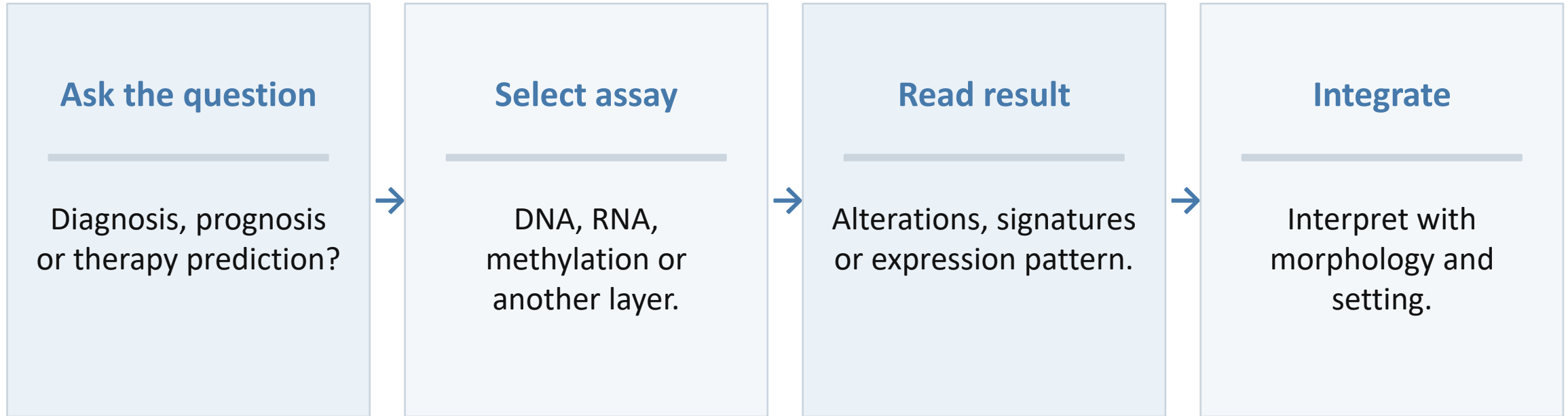
- Interrogates sequence alterations.
- Can reveal putative driver targets.
- Uses mutation and genome structure readouts.

Methylation profiling

- Measures epigenetic signatures.
- Can assist tumor classification.
- Source also notes potential prediction of therapy response.

The two methods can contribute complementary molecular classifications.

How to interpret a multi-omic profile



The best assay depends on what the clinician and pathologist need to know.

When a result is biologically informative

Detected

The method identifies a particular measured alteration.

Interpreted

Its relevance is considered in the correct neoplastic context.

Clinically used

The result informs diagnosis, outcome assessment or treatment discussion.

A detected alteration is not automatically a validated treatment decision.

Integrated pathology report: Two axes

Histopathology supplies	Molecular testing supplies	Joint interpretation
Lineage and tumor subtype	Alterations and molecular class	Refined diagnosis
Anaplasia and invasion	Relevant genetic signatures	Biologic risk context
Stroma and immune context	RNA / epigenetic readouts	Potential therapy implications

Clinical exercise: Diverse lung carcinoma

Case prompt A lung carcinoma has been diagnosed histologically. A clinician asks whether targeted therapy is plausible.

Reasoning

- The Robbins text emphasizes genetic diversity within lung carcinomas.
- An NGS panel may detect a relevant driver in an eligible tumor.
- The final result must retain histologic and clinical context.

Molecular profiling complements—not replaces—the established tumor diagnosis.

Clinical exercise: BRAF across tissues

Case prompt BRAF gain-of-function alterations are identified in three tumors of different histologic types.

Reasoning

- Heterogeneous responses to BRAF inhibitors.
- Hairy cell leukemia, melanoma and colon carcinoma behave differently.
- A shared target is not sufficient to assume the same benefit.

Report the precise genomic finding together with the histologic identity.

Clinical exercise: Repair-defect signature

Case prompt A tumor has a molecular pattern associated with mismatch repair deficiency.

Reasoning

- The genome-wide mutation pattern provides information beyond a single driver.
- MMR defects with checkpoint-blockade decisions.
- Therapeutic relevance can extend across anatomical tumor categories.

Recognize the repair-defect phenotype and its potential tumor-agnostic significance.

Clinical exercise: A breast RNA profile

Case prompt The treatment team requests an expression-based assessment of likely chemotherapy benefit in breast cancer.

Reasoning

- The task is predictive rather than merely diagnostic.
- RNA profiles capture gene-expression information.
- The excerpt provides no cutoff or specific assay eligibility rule.

Explain the intended clinical use while avoiding unsupported numerical thresholds.

Synthesis: Molecular plus morphologic

Find the lesion

Sequencing characterizes alterations and signatures.

Read the phenotype

Histopathology identifies type, invasion and anaplasia.

Understand the context

Tumor, stroma and immunity shape biological behavior.

Make a reasoned decision

A target is useful only after integrated clinical interpretation.

Source and reading

- Robbins, Cotran & Kumar. Pathologic Basis of Disease. 11th ed. Chapter 7, “Molecular Profiling of Tumors,” printed pp. 298–300.
- Figures 7.48–7.50: referenced for their documented concepts; original figures are not reproduced.
- Adjacent context: “Molecular Diagnostics and Cytogenetics,” printed pp. 297–298.
- Examples and schematics are educational reorganizations of these assigned passages.