

Immunohistochemistry

Tumor lineage, metastatic origin, and therapeutic biomarkers

From a difficult H&E diagnosis to a clinically useful protein-expression result

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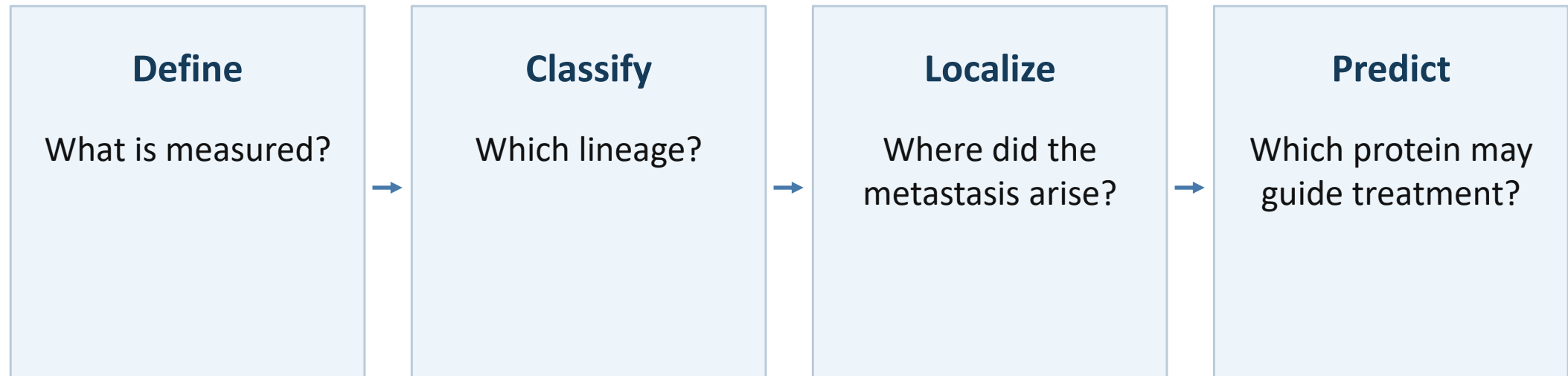
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Why morphology alone may be insufficient

- Routine hematoxylin and eosin sections establish the initial architecture and cytology.
- Severely anaplastic tumors may share overlapping appearances despite different lineages.
- A correct lineage diagnosis matters because prognosis and treatment differ.

Immunohistochemistry complements—not replaces—morphologic assessment.

Learning pathway



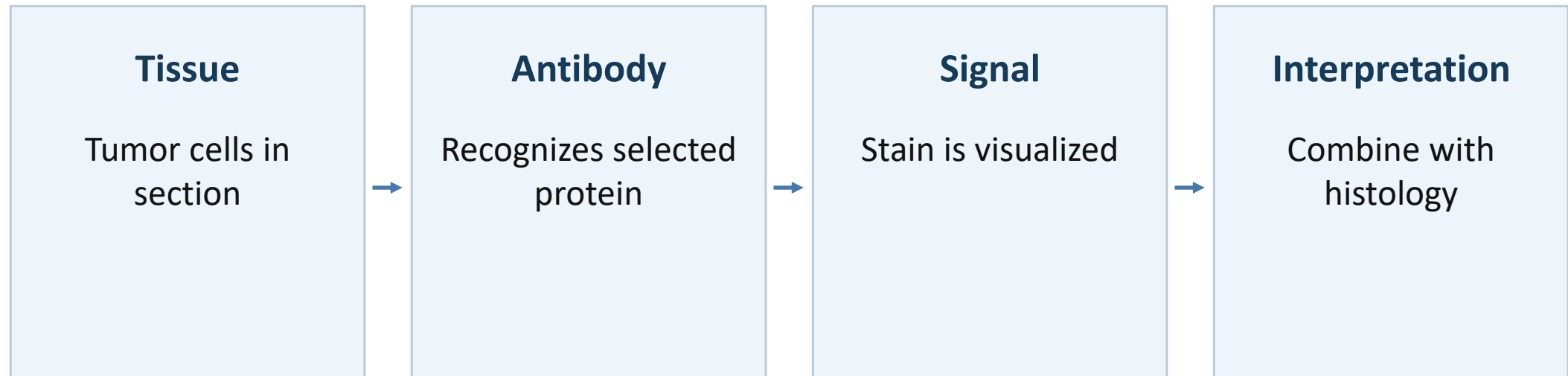
Keep one clinical question attached to every immunostain.

What immunohistochemistry measures

- Immunohistochemistry (IHC) applies antigen-specific antibodies to tissue sections.
- The reaction demonstrates expression of a selected protein within its tissue context.
- Markers can indicate differentiation, organ of origin, or a therapeutic target.

The measured feature is protein expression in a histologic section.

The antigen-to-interpretation chain



An antibody reaction becomes a diagnosis only after contextual interpretation.

Three distinct questions for one technique

Classification

What lineage does this poorly differentiated tumor resemble?

Examples: Cytokeratin, CD45, desmin, SOX10

Clinical stratification

Which organ or molecular target does the tumor suggest?

Examples: PSA, thyroglobulin, ER, PR, HER2, ALK

Marker selection follows the diagnostic question.

A positive stain is not a complete diagnosis

- A marker demonstrates a feature of a tumor—not its full identity.
- The clinical presentation, H&E appearance, and other marker results remain relevant.
- The best interpretation explains what the staining supports and what remains unresolved.

Use support-language rather than unsupported certainty.

Lineage markers versus organ-specific markers

Lineage-oriented

Resolve broad differentiation categories.

Cytokeratin → epithelial; CD45 → hematopoietic;
Desmin → muscle; SOX10 → melanocytic context

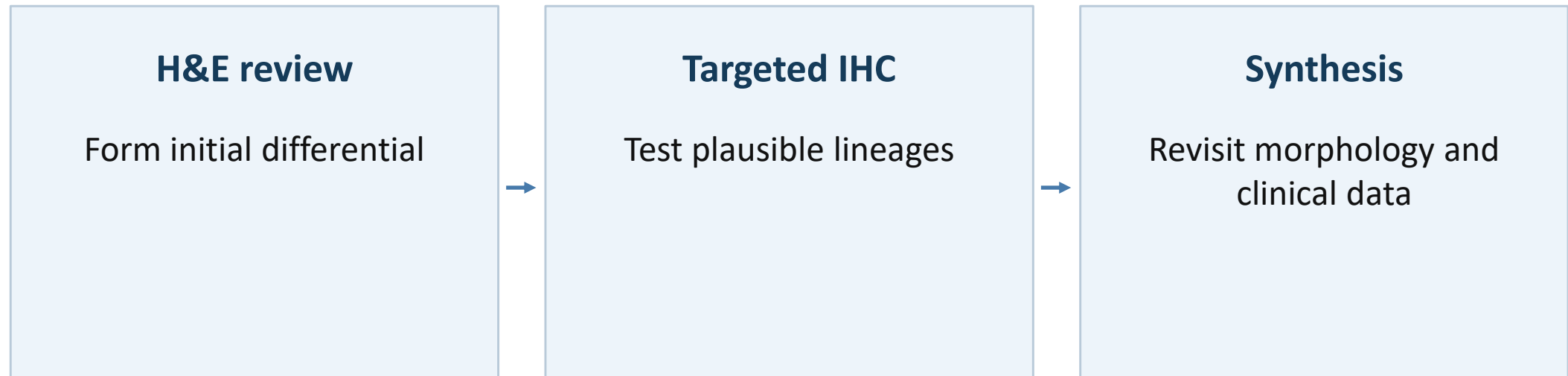
Organ-oriented

Refine a metastatic tumor's likely origin.

PSA → prostatic origin; Thyroglobulin → thyroid
follicular origin

Lineage and anatomic site are different levels of diagnostic inference.

The diagnostic path begins with H&E



Do not design a panel detached from the histologic differential.

Why undifferentiated tumors create a problem

- Loss of differentiation can erase characteristic tissue morphology.
- Carcinoma, lymphoma, melanoma, and sarcoma may be difficult to separate on H&E alone.
- These represent fundamentally different clinicopathologic groups.

The first useful IHC question is often broad lineage, not exact subtype.

Anaplastic tumor: Broad differential

Malignant neoplasm with limited differentiation

Carcinoma

Epithelial

Lymphoma

Hematopoietic

Melanoma

Melanocytic

Sarcoma

Mesenchymal / muscle

Initial categories direct the first-line protein-expression questions.

Why intermediate filaments are useful

- Intermediate-filament composition reflects cellular differentiation.
- Certain solid-tumor cells retain filaments associated with their cell of origin.
- IHC detection of these proteins can uncover otherwise invisible lineage information.

Protein phenotype can persist when microscopic differentiation is inconspicuous.

Cytokeratin: Evidence of epithelial lineage

Target

Cytokeratins are intermediate-filament proteins of epithelial cells.

Inference

Tumor-cell staining supports carcinoma in an appropriate morphologic setting.

Cytokeratin is a lineage clue, not an automatic site-of-origin diagnosis.

Desmin: A muscle-differentiation clue

Target

Desmin is an intermediate-filament protein associated with muscle differentiation.

Inference

Tumor-cell expression supports the muscle branch of a differential.

Interpret desmin as part of the complete phenotype and morphology.

CD45: Hematopoietic differentiation

Target

CD45 is a major marker of hematopoietic cells and neoplasms.

Inference

Staining in an anaplastic tumor supports a hematolymphoid category.

A hematolymphoid result changes the subsequent diagnostic work-up.

SOX10: A melanocytic clue

Target

SOX10 is a transcription factor expressed by most melanomas.

Inference

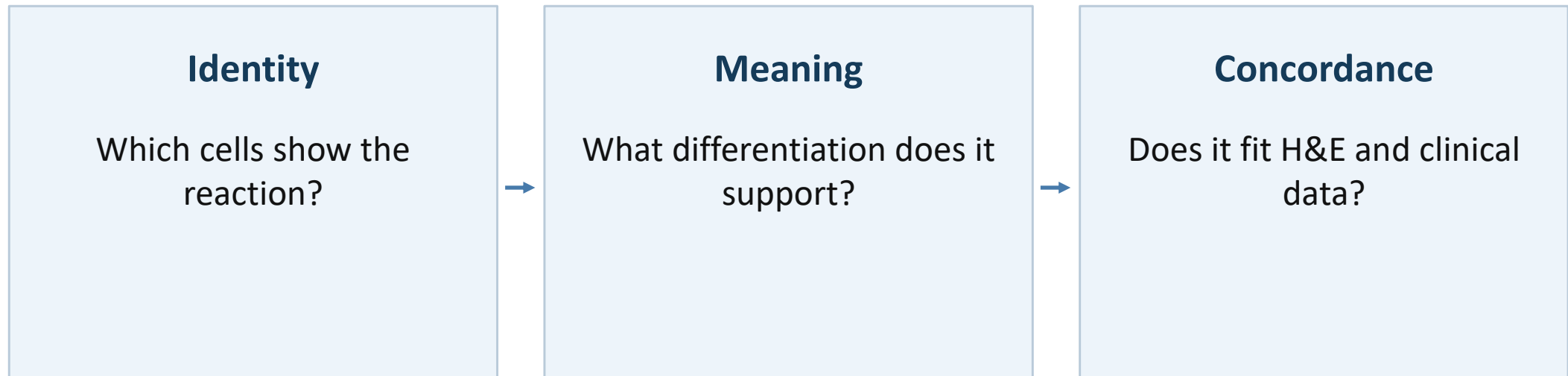
SOX10 expression supports consideration of melanoma in the right differential.

“Most” is not “all,” and expression is not equivalent to a complete diagnosis.

First-line lineage comparison

Marker	Protein / role	Main inference
Cytokeratin	Epithelial intermediate filaments	Carcinoma lineage
Desmin	Muscle intermediate filament	Muscle differentiation
CD45	Hematopoietic antigen	Hematolymphoid lineage
SOX10	Transcription factor	Melanoma in context

Marker interpretation: Three questions



Tumor-cell identity, inference, and concordance are separate checks.

Broad lineage and tumor subtyping

First decision

Resolve carcinoma versus hematolymphoid, melanocytic, or other lineage.

Use broad markers aligned to the H&E differential.

Later decisions

Refine tumor type, tissue of origin, and therapeutic phenotype.

Use narrower markers only after the broad differential is organized.

A staged work-up preserves the purpose of every test.

Illustrative case: Undifferentiated mass

Illustrative case — constructed teaching example

A biopsy shows a malignant tumor with no convincing epithelial, lymphoid, or melanocytic features on H&E.

What should the first IHC panel aim to resolve?

Broad cellular lineage

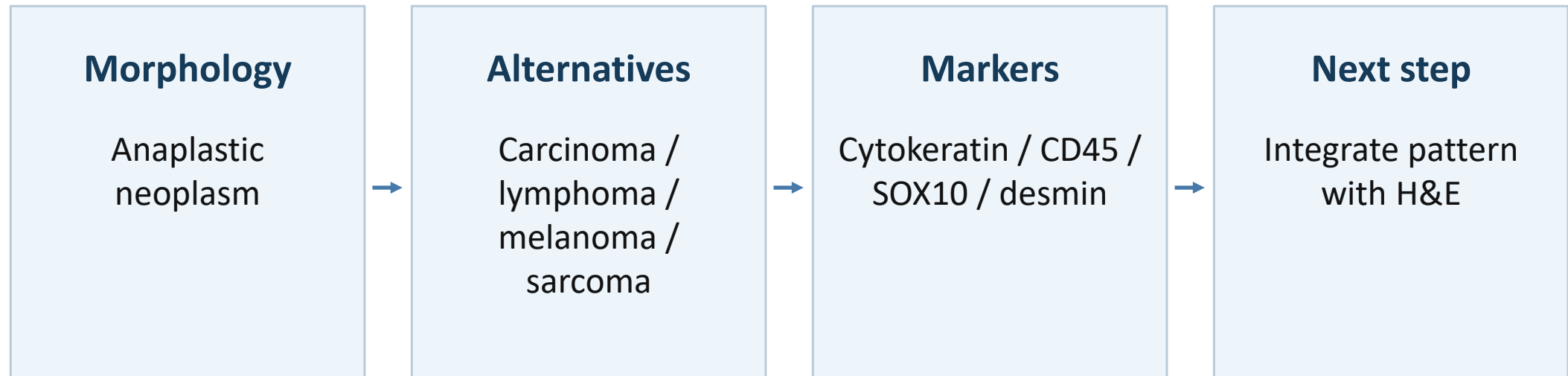
Exact primary site immediately

HER2 status before lineage

Reasoning

Lineage separation as a central application of IHC in anaplastic tumors.

Case reasoning: Broad lineage panel



This is an illustrative decision sequence, not a rigid universal panel.

Illustrative case: Cytokeratin-positive tumor

Illustrative case — constructed teaching example

A poorly differentiated malignant tumor shows convincing tumor-cell cytokeratin expression.

Which initial conclusion is justified?

**Supports epithelial
differentiation**

Proves a specific anatomic
primary

Proves sensitivity to anti-HER2
therapy

Reasoning

Cytokeratin is used to infer epithelial lineage; Organ and treatment are separate questions.

Illustrative case: CD45-positive mass

Illustrative case — constructed teaching example

A malignant tumor of uncertain type shows CD45 expression in the tumor-cell population.

What diagnostic branch becomes particularly important?

Hematolymphoid neoplasm

Thyroid follicular carcinoma

HER2-amplified breast carcinoma

Reasoning

CD45 expression supports hematopoietic lineage; Further classification may require additional tests.

Illustrative case: SOX10-positive tumor

Illustrative case — constructed teaching example

An anaplastic tumor has a protein-expression pattern that includes SOX10.

What is the appropriately qualified interpretation?

Melanoma enters the favored differential

Every SOX10-positive tumor is melanoma

Carcinoma is proven

Reasoning

SOX10 expression in most melanomas, but interpretation remains contextual.

Illustrative case: Desmin-positive tumor

Illustrative case — constructed teaching example

A malignant spindle-cell tumor expresses desmin in the neoplastic cells.

Which differentiation is being suggested?

Muscle differentiation

Hematopoietic origin

HER2 amplification

Reasoning

Desmin provides an intermediate-filament clue to muscle origin.

Why lineage identification matters clinically

- The potential tumor groups have different biological behavior.
- Management is not interchangeable across carcinoma, lymphoma, melanoma, and sarcoma.
- IHC can make a morphologically ambiguous specimen clinically actionable.

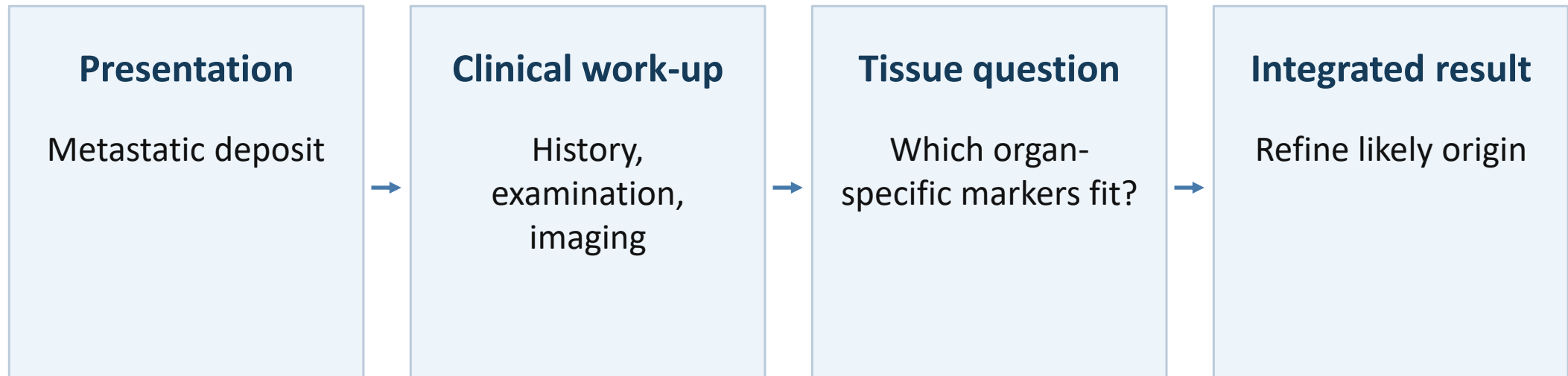
The value of a lineage stain depends on whether it changes the classification.

The metastatic lesion may arrive first

- Patients can present with metastatic cancer before the primary tumor is known.
- Clinical assessment and imaging may identify the origin, but sometimes remain inconclusive.
- IHC can search for tissue-associated antigens within the metastatic biopsy.

The metastatic biopsy can carry clues to the tumor's original tissue.

Unknown-primary reasoning sequence



IHC contributes to—but does not replace—the clinical search.

PSA: A clue to prostatic origin

Protein

Prostate-specific antigen (PSA) can be detected in tumor tissue by IHC.

Inference

In a metastatic carcinoma, an appropriate positive result may identify a prostatic source.

Tissue PSA staining is not the same task as serum PSA monitoring.

Thyroglobulin: A clue to thyroid origin

Protein

Thyroglobulin is a tissue-associated protein used for thyroid-origin assessment.

Inference

Staining in a metastatic carcinoma can support thyroid follicular origin.

Choose markers according to the plausible organs and morphology.

From lineage to organ of origin

Clinical question	Representative marker	Interpretive level
Is the tumor epithelial?	Cytokeratins	Broad epithelial lineage
Is prostate an origin?	PSA	Prostate-associated antigen
Is thyroid an origin?	Thyroglobulin	Thyroid-associated antigen

Illustrative metastasis: PSA expression

Illustrative case — constructed teaching example

A metastatic carcinoma has an uncertain origin. The tumor cells show PSA expression.

Which statement is best supported?

A prostatic primary is suggested

This proves a thyroid primary

Tumor lineage is hematopoietic

Reasoning

PSA staining as a means of identifying prostatic carcinoma among metastases.

Illustrative metastasis: Thyroglobulin

Illustrative case — constructed teaching example

A metastatic carcinoma is being evaluated for a possible thyroid primary. Tumor cells stain for thyroglobulin.

How should the result be used?

Support a thyroid source in context

Treat as a measure of ALK activity

Ignore the histologic appearance

Reasoning

Thyroglobulin is the source example of an organ-associated antigen in metastatic diagnosis.

Histology and IHC answer different questions

Histology

Recognizes architecture, cytologic features, and tissue relationships.

May suggest malignancy even without recognizable lineage.

IHC

Shows selected proteins within the examined tissue.

May clarify lineage, likely primary, or clinical target.

A coherent final diagnosis synthesizes both evidence streams.

Clinical biomarkers: Three distinct roles

- Diagnostic: Helps define tumor identity or origin.
- Prognostic: Associated with expected disease behavior or outcome.
- Predictive: Identifies potential response to a particular therapy.

A marker may serve more than one role, but the meanings must not be conflated.

ER and PR expression in breast cancer

Test

IHC detects estrogen receptor (ER) and progesterone receptor (PR) expression in tumor cells.

Clinical use

The hormone-receptor phenotype has prognostic and therapeutic relevance.

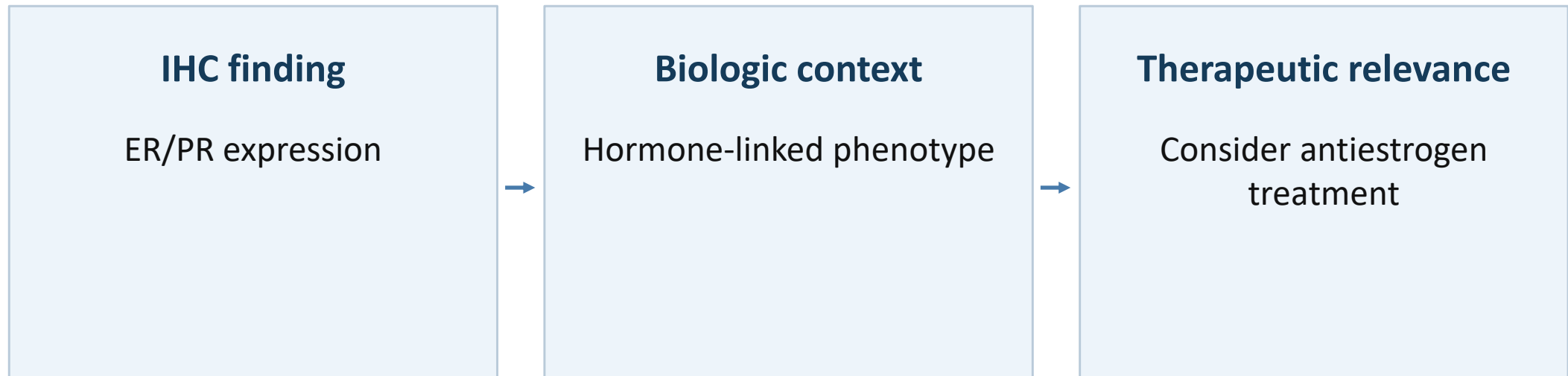
This example moves beyond lineage into a treatment-linked phenotype.

Hormone receptors and prognosis

- Receptor-positive breast cancers generally have a better prognosis than receptor-negative cancers.
- “Generally” describes a broad association rather than an individual patient prediction.
- Clinical interpretation remains integrated with the remainder of tumor assessment.

Prognostic information refers to expected disease behavior.

Hormone receptors and therapeutic reasoning



A predictive biomarker links a biological feature to a treatment option.

Prognostic versus predictive: ER/PR

Prognostic statement

Receptor-positive breast cancers generally show more favorable outcomes than receptor-negative tumors.

Predictive statement

Hormone-receptor expression provides a rationale for antiestrogen therapy.

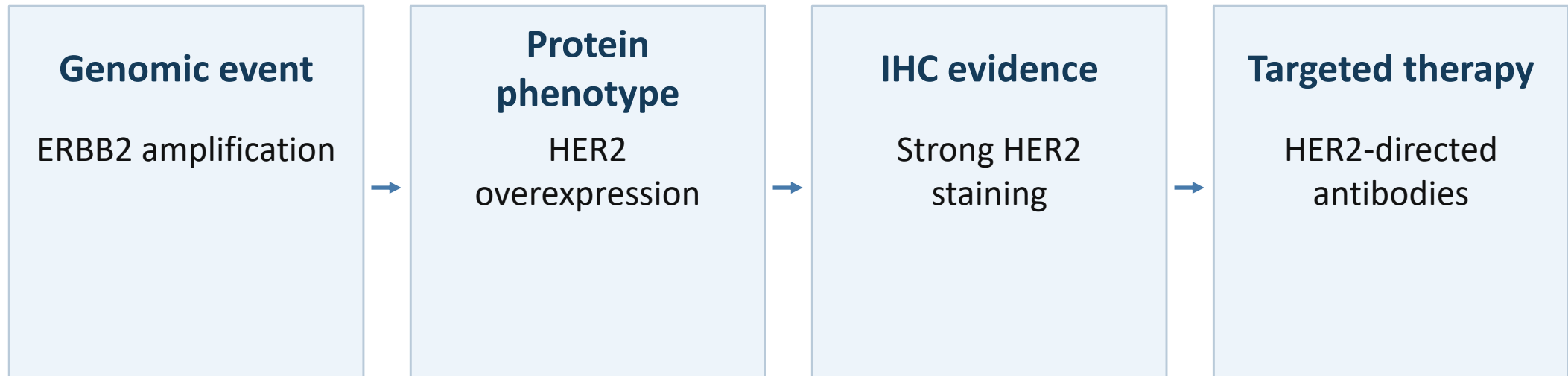
One result can inform both categories without making them synonymous.

HER2 and the ERBB2 gene

- ERBB2 is the gene encoding the HER2 receptor tyrosine kinase.
- In some breast carcinomas, ERBB2 amplification produces high HER2 protein expression.
- IHC assesses the protein, whereas in situ hybridization can assess gene amplification.

The protein assay and genomic assay address related but distinct analytes.

HER2: Gene-to-protein-to-treatment



This is the biologic chain described for a subset of breast cancers.

HER2 IHC and FISH are complementary

Immunohistochemistry

What is assessed? HER2 protein expression in tumor tissue.

Output: Protein-staining interpretation.

Fluorescence in situ hybridization

What is assessed? ERBB2 gene amplification.

Output: Gene-copy evidence.

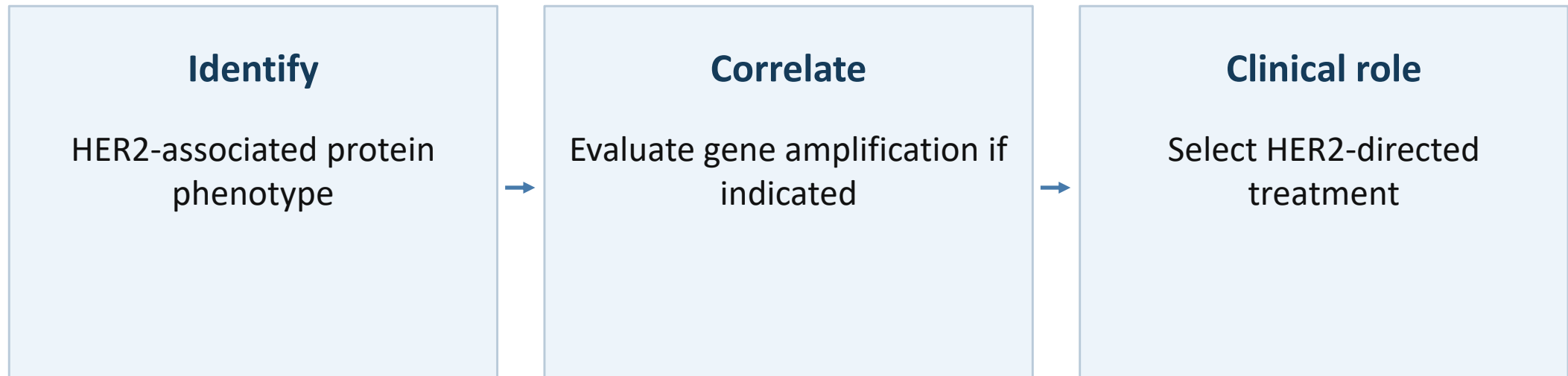
FISH as an adjunct when confirmation is needed.

Why FISH may add information

- HER2 protein overexpression and ERBB2 amplification are related biological events.
- A protein-expression result is not identical to a direct gene assay.
- The two techniques can therefore provide complementary evidence.

IHC characterizes protein expression; FISH assesses ERBB2 amplification.

HER2 as a predictive biomarker



The central predictive link is between a molecular target and a treatment.

Illustrative case: Strong HER2 staining

Illustrative case — constructed teaching example

A breast carcinoma shows strong HER2 protein staining. A request is made to evaluate ERBB2 amplification.

What is the reason for adding an in situ hybridization assay?

To assess the associated gene-level event

To detect desmin filaments

To establish hematopoietic lineage

Reasoning

FISH can confirm ERBB2 amplification as a complement to protein IHC.

ALK protein as a therapeutically relevant clue

Molecular context

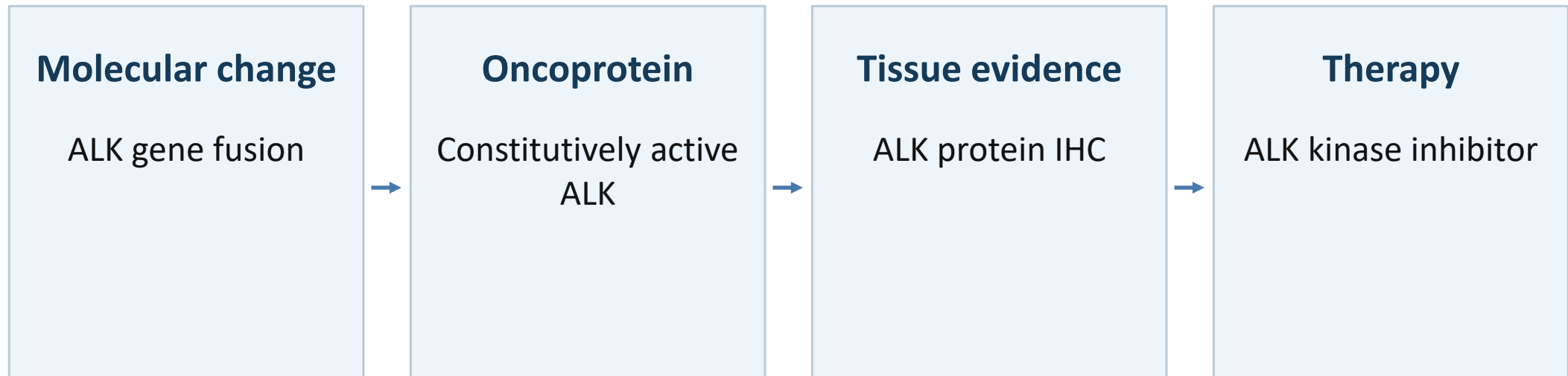
Some tumors express constitutively active ALK fusion proteins.

Assay and consequence

ALK IHC may identify such tumors for consideration of ALK tyrosine kinase inhibition.

A protein result can point toward an actionable kinase alteration.

ALK activation and targeted therapy



The source describes ALK-driven lung cancers and lymphomas.

ALK-positive lung cancer context

- A subset of lung carcinomas contains ALK fusion proteins.
- An EML4–ALK fusion as one molecular example.
- Recognition of activated ALK has therapeutic implications because ALK can be inhibited.

The relevant chain is fusion → active kinase → therapeutic target.

ALK-positive lymphoma context

- The textbook also identifies ALK-expressing lymphomas.
- ALK expression can therefore be clinically relevant in more than one tumor lineage.
- Tumor type must be established before interpreting an ALK result as a therapeutic phenotype.

One target can appear across distinct histologic categories.

Clinical roles of the representative markers

Marker	Main question	Use emphasized here
Cytokeratin / CD45	What is the lineage?	Diagnostic
PSA / thyroglobulin	Where might it arise?	Diagnostic
ER / PR	Prognosis and endocrine response?	Prognostic + predictive
HER2 / ALK	Is a target expressed?	Predictive / treatment linked

IHC does not directly measure every alteration

Protein-based readout

Cytokeratin, desmin, CD45, SOX10, ER/PR, HER2, ALK.

Information about expression and phenotype.

Gene-level readout

ERBB2 amplification by FISH; ALK rearrangement is a genomic event.

Molecular mechanism may require a complementary test.

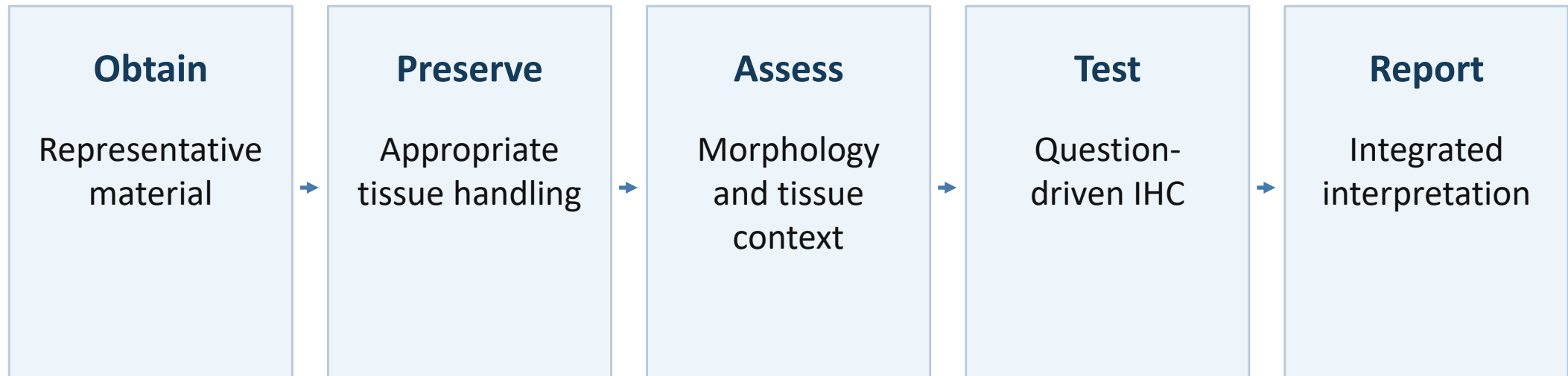
Do not equate protein positivity with a specific genomic event in every case.

Specimen adequacy precedes assay interpretation

- The source emphasizes representative sampling and proper preservation.
- An unrepresentative specimen can misdirect both morphologic and ancillary interpretation.
- The tumor-cell population must be identified within the tested material.

Better marker selection cannot rescue the wrong sampled tissue.

Specimen-to-report quality chain



The quality of the final inference depends on every earlier stage.

What a disciplined IHC interpretation says

- State which relevant tumor cells express the selected antigen.
- Explain the differential diagnosis supported by that result.
- Distinguish a lineage inference from an organ inference or therapy implication.
- Acknowledge unresolved alternatives rather than claiming certainty from one marker.

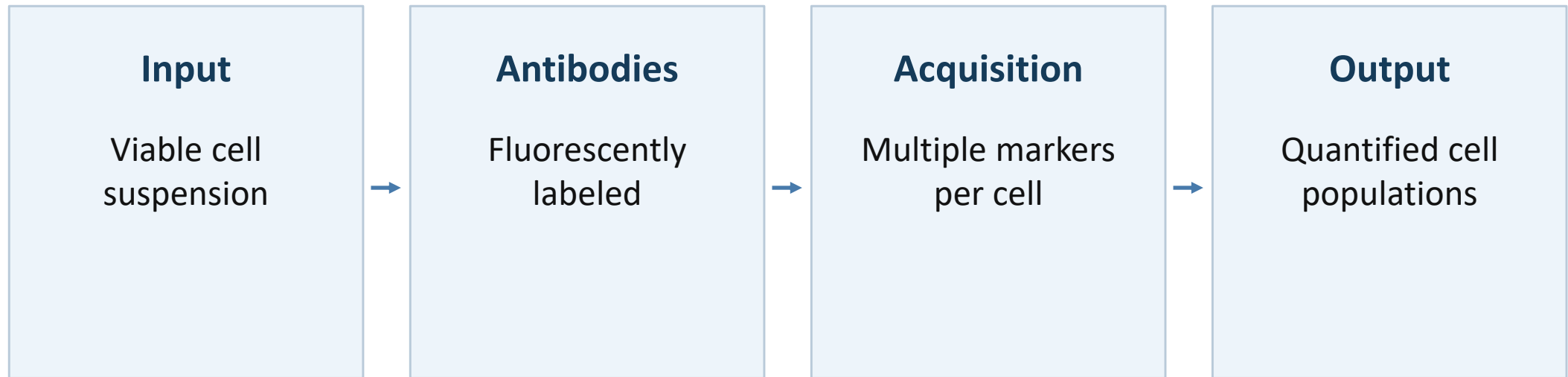
The report should answer the clinical question, not merely list positive stains.

Flow cytometry: The adjacent method

- Flow cytometry measures cellular properties rapidly and quantitatively.
- It analyzes viable cells maintained in suspension.
- It is used particularly for leukemias and lymphomas arising from hematopoietic tissues.

Its specimen requirements differ fundamentally from tissue-section IHC.

How flow cytometry builds a phenotype



The ability to measure several antigens on the same cell is a central advantage.

IHC versus flow cytometry

Immunohistochemistry

Works on tissue sections.

Retains architectural context.

Flow cytometry

Requires viable cell suspension.

Quantifies several antigens on individual cells simultaneously.

Different specimen formats generate complementary information.

Illustrative case: A suspected lymphoma

Illustrative case — constructed teaching example

A tumor is suspicious for a hematopoietic neoplasm, and material suitable for viable-cell suspension is available.

What does flow cytometry add beyond routine section IHC?

Simultaneous multiparameter antigen analysis

Microscopic tissue architecture

Direct measurement of ERBB2 amplification

Reasoning

The source specifically contrasts flow cytometry's multiparameter per-cell readout with IHC.

When tissue architecture is essential

Section-based IHC

Locate an antigen in the tumor and adjacent tissue.

Correlate stain with the morphological region under review.

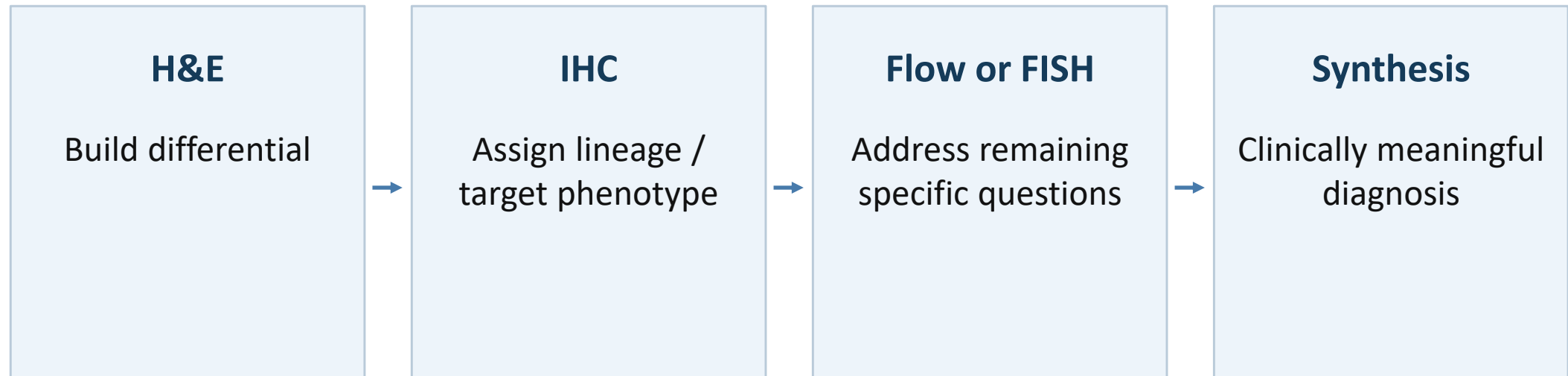
Cell suspension

Characterize populations without preserving the original tissue architecture.

Simultaneously measure multiple fluorescence-labeled antigens.

Architecture and multidimensional cellular phenotype answer different questions.

Combined testing for a difficult neoplasm



Ancillary methods are chosen for a stated unresolved question.

Integrated case: Lineage then treatment

Illustrative case — constructed teaching example

An anaplastic neoplasm expresses cytokeratin. It is classified as carcinoma, and an organ-specific biomarker is then considered.

Why is the order of these decisions useful?

**Classification and target
assessment are different tasks**

Any cytokeratin-positive tumor
must express HER2

Imaging is never relevant

Reasoning

The workflow avoids conflating lineage, organ origin, and predictive phenotype.

Integrated case: Uncertain primary

Illustrative case — constructed teaching example

A metastatic carcinoma is morphologically ambiguous and the clinical search has not established a primary. PSA and thyroglobulin are considered.

What determines the meaning of the results?

Tumor staining plus clinical and morphologic context

The antibody name alone

Only whether the lesion is large

Reasoning

The source describes site inference from tissue-specific antigens in biopsies of metastases.

Integrated case: Breast biomarkers

Illustrative case — constructed teaching example

A breast carcinoma is evaluated with ER/PR and HER2 IHC as part of a clinically relevant protein-expression assessment.

Which statement best distinguishes the assays?

ER/PR informs endocrine relevance; HER2 informs receptor-targeted relevance

Both markers prove a prostate primary

Neither can affect treatment planning

Reasoning

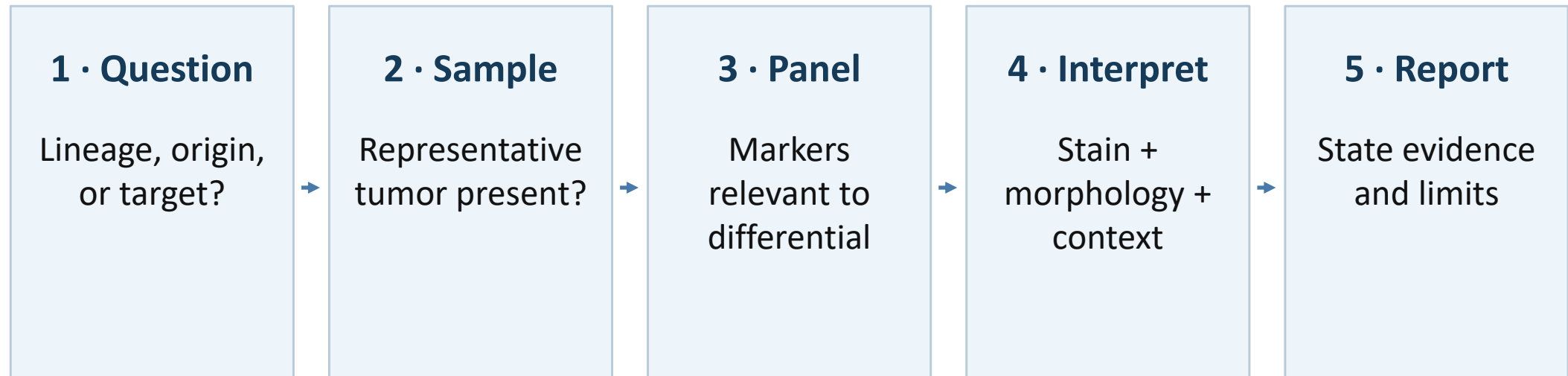
Robbins assigns therapeutic relevance to both hormone receptors and HER2 through different treatment mechanisms.

Five interpretive errors to avoid

- Calling a broad lineage marker an organ-specific proof.
- Equating a biomarker's prognostic role with its predictive role.
- Confusing HER2 protein expression with direct ERBB2 amplification measurement.
- Ignoring specimen quality or discordant morphology.
- Using a single illustrative marker as a universal diagnostic rule.

IHC is most reliable as a hypothesis-driven component of diagnosis.

A practical IHC decision framework



One workflow spans all three clinical applications emphasized.

Concept synthesis: Marker to inference

Observed feature	Best initial inference	Do not conclude alone
Cytokeratin	Epithelial differentiation	Specific primary site
CD45	Hematopoietic lineage	Exact lymphoma subtype
PSA	Possible prostate source	Complete origin work-up
ER/PR	Hormone-receptor phenotype	Guaranteed treatment response
HER2	Target-related protein expression	Direct gene-copy count

Core messages

- Morphology establishes the problem; IHC tests a specific diagnostic or therapeutic hypothesis.
- Broad lineage markers help classify anaplastic tumors; Organ-associated markers can suggest an occult primary.
- ER/PR, HER2, and ALK show how tumor protein expression can guide clinical decisions.
- Flow cytometry and in situ hybridization contribute different, complementary information.

Interpret staining as evidence—with its scope and limits stated.

Source and scope

- Robbins, Cotran & Kumar. Pathologic Basis of Disease. 11th ed. Chapter 7: Neoplasia, “Immunohistochemistry,” p. 297.
- Adjacent Chapter 7: “Histologic and Cytologic Methods”; “Flow Cytometry”; “Molecular Diagnostics and Cytogenetics,” pp. 296–298.
- Related Robbins context: Chapter 7 (oncogenic growth-factor receptors, p. 253); Chapter 23 (breast cancer biomarkers, pp. 959–960).

Constructed cases and visual diagrams are teaching schematics; no case represents an actual specimen or patient.