

# Molecular Diagnostics and Cytogenetics

Diagnostic classification · prognostic stratification · molecular monitoring · precision therapy

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# Learning objectives

- Select an assay that detects the relevant class of genetic alteration.
- Explain the diagnostic role of clonality, translocations, FISH, PCR, arrays, and sequencing.
- Distinguish prognostic, predictive, and residual-disease applications.
- Integrate germline testing and liquid biopsy with morphologic assessment.

# Molecular testing is an adjunct to pathology

- Histology and cytology establish most cancer diagnoses.
- Molecular methods add decisive information in selected diagnostic problems.
- A result is interpreted in relation to specimen, morphology, and clinical question.
- The same molecular lesion may carry different implications in different tumor contexts.

# Six clinical questions for a molecular laboratory

**What is the  
tumor?**



How may it  
behave?



Is disease still  
present?



Is risk  
inherited?



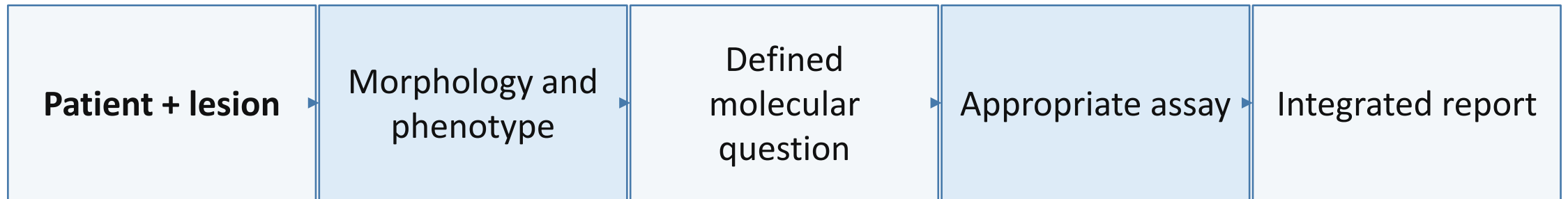
Is a target  
present?



Has resistance  
emerged?

Diagnosis · prognosis · monitoring · predisposition · treatment · resistance

# Start with the clinical question, not the technology



A test is useful only when its analytical target answers the diagnostic or therapeutic question.

# Biologic material determines feasible testing

| Material                    | Advantages                            | Important considerations                    |
|-----------------------------|---------------------------------------|---------------------------------------------|
| <b>Tumor tissue</b>         | Morphology and tumor-specific DNA/RNA | Tumor fraction and fixation affect analysis |
| <b>Blood / marrow cells</b> | Accessible hematologic tumor material | Cell population must reflect the lesion     |
| <b>Cell-free plasma DNA</b> | Serial, minimally invasive sampling   | Detected material may be sparse             |

## Molecular lesion classes require different methods

| Alteration                    | Illustrative detection methods           |
|-------------------------------|------------------------------------------|
| Point mutation                | PCR / sequence-based assays              |
| Gene fusion / translocation   | Cytogenetics, FISH, RT-PCR or sequencing |
| Copy-number gain or loss      | FISH, arrays or sequence-based assays    |
| Clonal receptor rearrangement | PCR for IG or TCR rearrangements         |

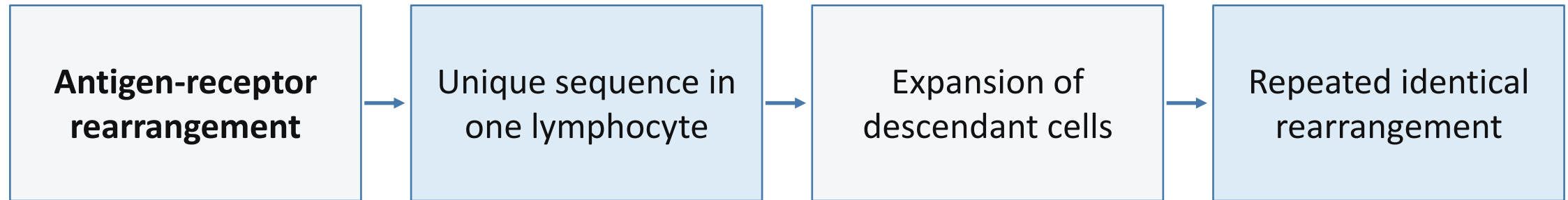
## Assay selection: What can each technique answer?

| Technique                 | Most direct readout              | Typical clinical use                   |
|---------------------------|----------------------------------|----------------------------------------|
| <b>PCR / RT-PCR</b>       | Defined DNA or RNA sequence      | Clonality, fusions, disease burden     |
| <b>Karyotyping</b>        | Large-scale chromosomal change   | Characteristic acquired rearrangement  |
| <b>FISH</b>               | Targeted chromosomal interval    | Fusion/rearrangement or amplification  |
| <b>SNP / tiling array</b> | Genome-wide copy number          | Deletions, gains and allelic imbalance |
| <b>Targeted NGS</b>       | Sequences in many selected genes | Molecular disease subclassification    |

# Clonality is a diagnostic question in lymphoid lesions

- Neoplastic B- and T-cell proliferations arise from a single founding clone.
- That clone carries a characteristic antigen-receptor gene rearrangement.
- Reactive lymphoid tissue contains many clones with distinct rearrangements.
- PCR analysis can therefore help distinguish clonal and polyclonal proliferations.

# Why antigen-receptor rearrangements act as clonal markers



Schematic: Shared rearrangement acts as a molecular fingerprint of a lymphoid clone.

# Immunoglobulin gene rearrangement testing

- B-cell antigen-receptor loci undergo somatic rearrangement.
- PCR targets rearranged immunoglobulin gene sequences.
- A restricted clonal pattern supports a B-cell clonal expansion.
- The assay contributes to, but does not replace, full lymphoma assessment.

## T-cell receptor gene rearrangement testing

- Each T-cell clone carries characteristic rearranged TCR gene sequences.
- PCR provides evidence of a dominant T-cell population.
- A reactive infiltrate is expected to include numerous different T-cell clones.
- Interpretation requires knowledge of the cellular context of the sample.

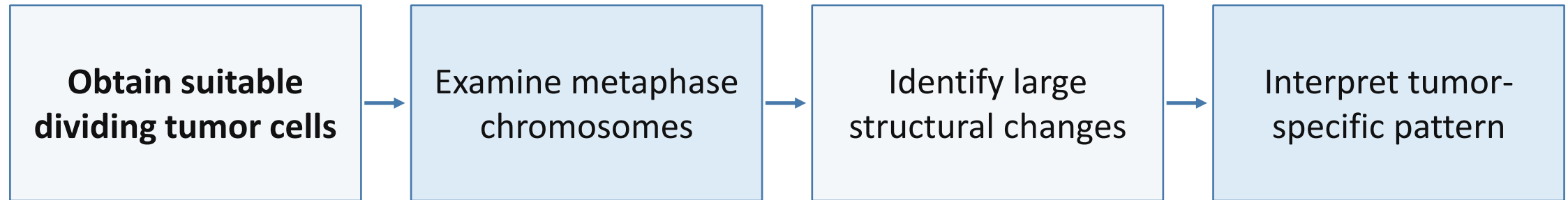
# Clonality and malignancy are not synonymous questions

| Molecular result             | Supported interpretation               | What remains necessary                          |
|------------------------------|----------------------------------------|-------------------------------------------------|
| <b>Clonal pattern</b>        | Dominant related lymphocyte population | Morphology, phenotype and clinical context      |
| <b>Polyclonal pattern</b>    | Multiple lymphocyte populations        | Assessment of sampling and diagnostic suspicion |
| <b>Inconclusive analysis</b> | No definitive clonal assignment        | Alternative or complementary evaluation         |

## Recurrent translocations are tumor-associated markers

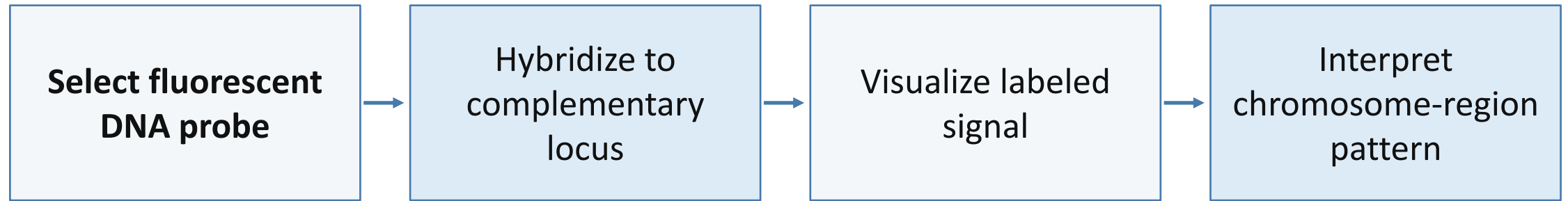
- Many leukemias and lymphomas harbor characteristic chromosomal translocations.
- A rearrangement may create a fusion oncogene or dysregulate a gene.
- Routine cytogenetics and FISH can demonstrate selected abnormalities.
- Finding a characteristic lesion can substantially refine tumor classification.

# What routine cytogenetics contributes



Conventional analysis surveys the karyotype; Obtaining metaphases can be difficult in some solid tumors.

# FISH: Target-specific hybridization



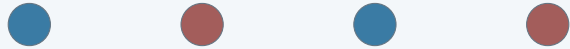
Schematic method; FISH is applicable to metaphase preparations and interphase nuclei.

# Metaphase and interphase FISH

| Preparation                  | Useful feature                                      | Constraint                                              |
|------------------------------|-----------------------------------------------------|---------------------------------------------------------|
| <b>Metaphase chromosomes</b> | Chromosomal context for a target locus              | Depends on available metaphases                         |
| <b>Interphase nuclei</b>     | Examines nondividing nuclei, including fixed tissue | Probe signal must be interpreted without full karyotype |

# FISH probe configurations are question-specific

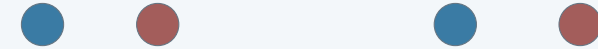
## Locus copy number



Example: Increased locus signals

More locus signals can indicate amplification; Interpretation requires appropriate controls.

## Break-apart strategy



Example: Spatially separated probe components

Separated signal components can indicate rearrangement involving the interrogated locus.

Schematic only — not a patient FISH micrograph

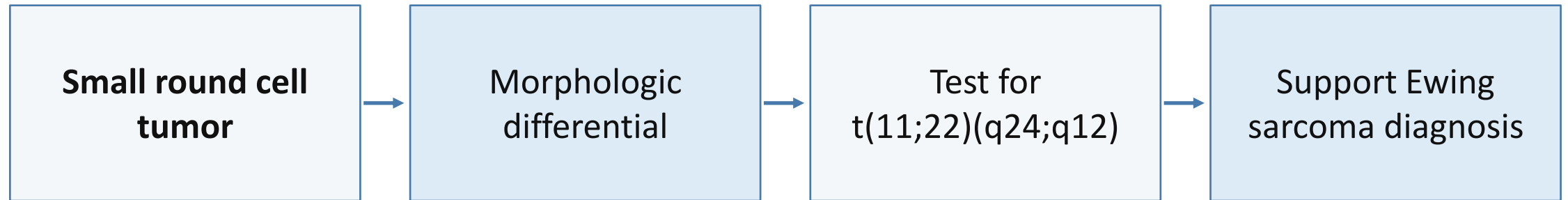
# Chromosomal rearrangements: Method trade-offs

| Method           | Strength                                    | Question to ask                       |
|------------------|---------------------------------------------|---------------------------------------|
| <b>Karyotype</b> | Broader structural chromosome survey        | Can tumor metaphases be obtained?     |
| <b>FISH</b>      | Targeted locus analysis in cells or tissue  | Which rearrangement is suspected?     |
| <b>RT-PCR</b>    | Detection of a particular fusion transcript | Is its expressed transcript testable? |

## Round blue cell tumors: Morphology may be insufficient

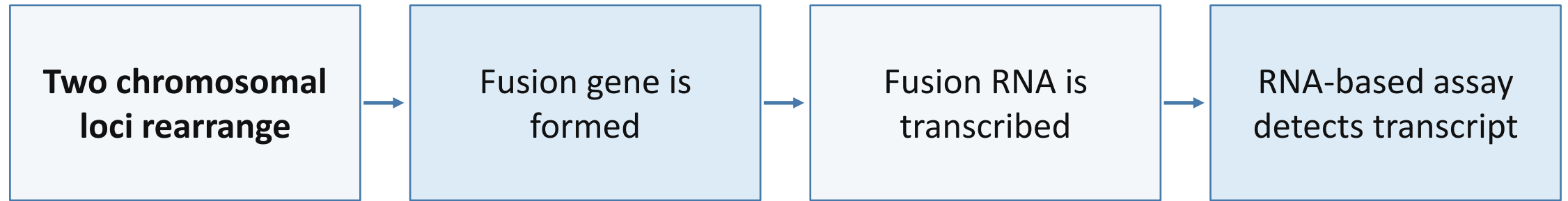
- Several childhood sarcomas show overlapping small round cell morphology.
- A specific chromosomal rearrangement may distinguish a particular entity.
- Molecular findings are combined with the morphologic differential diagnosis.
- This is especially valuable when preparation of tumor chromosomes is difficult.

# Ewing sarcoma: A characteristic translocation



The characteristic rearrangement is a confirmatory molecular clue—not a substitute for tumor classification.

# A translocation can generate a fusion transcript

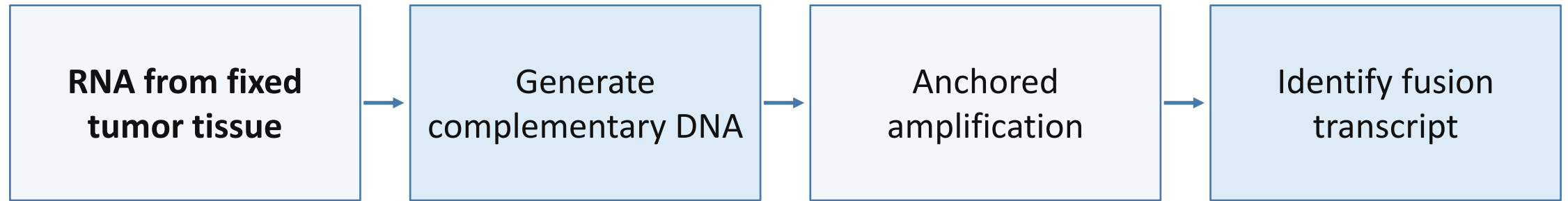


Schematic principle; The molecular target can be DNA rearrangement or expressed fusion RNA.

## PCR and reverse-transcription PCR are distinct

| PCR                                    | RT-PCR                                    |
|----------------------------------------|-------------------------------------------|
| Starts with DNA template               | Starts with RNA; Makes complementary DNA  |
| Amplifies a selected DNA locus         | Amplifies a selected expressed RNA target |
| Examples: Receptor gene rearrangements | Examples: BCR-ABL fusion transcripts      |

# Anchored RT-PCR expands fusion transcript detection



Anchored RT-PCR as particularly useful with formalin-fixed paraffin-embedded tissue.

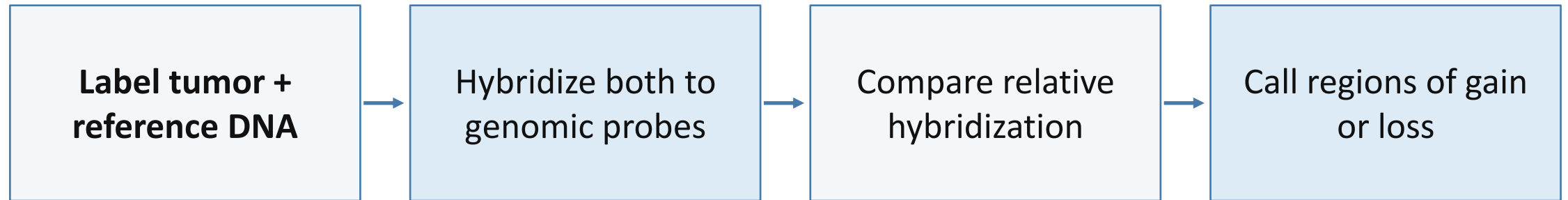
## Fixed tissue expands access—and complicates RNA assays

- Formalin-fixed, paraffin-embedded tumor tissue is widely available.
- Anchored RT-PCR can identify a broad range of tumor-associated fusion transcripts.
- RNA is comparatively prone to degradation during tissue processing.
- Method selection must account for the available analyte and preservation.

## Arrays survey copy-number changes across the genome

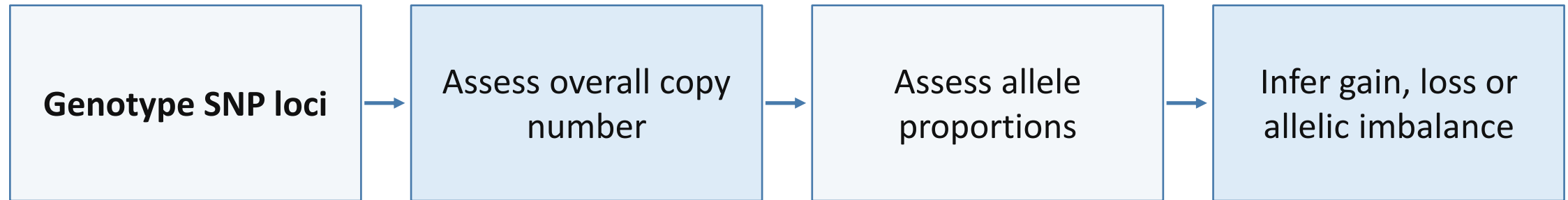
- DNA microarrays can map deletions and amplifications at multiple loci.
- Tiling designs sample regions distributed throughout the genome.
- SNP arrays also provide information about polymorphic alleles.
- Genome-wide arrays differ from a FISH assay directed at one or a few loci.

# Array comparative genomic hybridization



Schematic; Relative signal imbalance indicates copy-number change, not a patient measurement.

## SNP arrays add allele-specific information



SNP markers can reveal loss of heterozygosity in addition to copy-number abnormalities.

# Copy-number loss versus loss of heterozygosity

| Finding                       | What is observed                         | Interpretive point                               |
|-------------------------------|------------------------------------------|--------------------------------------------------|
| <b>Copy-number loss</b>       | Reduced quantity of DNA at a locus       | Deletion is one potential mechanism              |
| <b>Loss of heterozygosity</b> | Loss of mixed allelic state              | May occur even with retained diploid copy number |
| <b>Both together</b>          | Reduced DNA amount and allelic diversity | Concordant pattern supports an allelic deletion  |

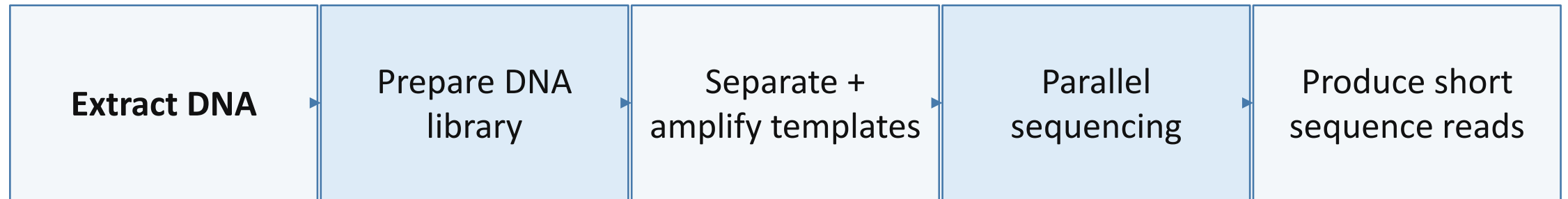
## FISH and arrays answer different scales of question

| FISH                                                        | Genomic array                                       |
|-------------------------------------------------------------|-----------------------------------------------------|
| Interrogates selected chromosome regions                    | Surveys many intervals genome-wide                  |
| Displays signals within nuclei or chromosomes               | Reports locus-level hybridization/genotype patterns |
| Supports targeted rearrangement or amplification evaluation | Maps regional gains, losses and allelic changes     |

## NGS permits broad molecular characterization

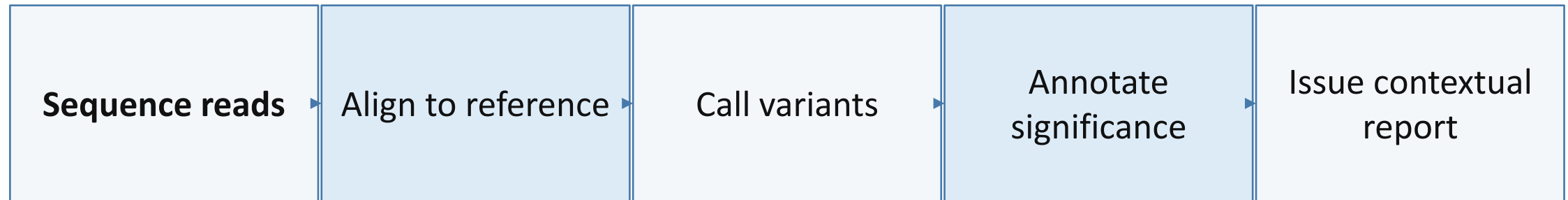
- Next-generation sequencing reads many DNA molecules in parallel.
- Tumor genomes can contain numerous simultaneous alterations.
- Targeted sequencing enables evaluation of selected panels of cancer-relevant genes.
- NGS increasingly contributes to molecular classification, especially in hematologic disease.

# NGS: Biological sample to sequence reads



Simplified schematic of a common massively parallel short-read sequencing approach.

# NGS bioinformatics produces clinically interpretable variants



Depth, allele fraction, annotation and the clinical setting determine interpretability.

## Sequencing depth affects variant detection

- Depth denotes the number of reads covering a genomic location.
- Greater depth can support identification of lower-frequency variants.
- Variant allele frequency describes the proportion of reads carrying a variant.
- Low-frequency variation requires careful analytical interpretation.

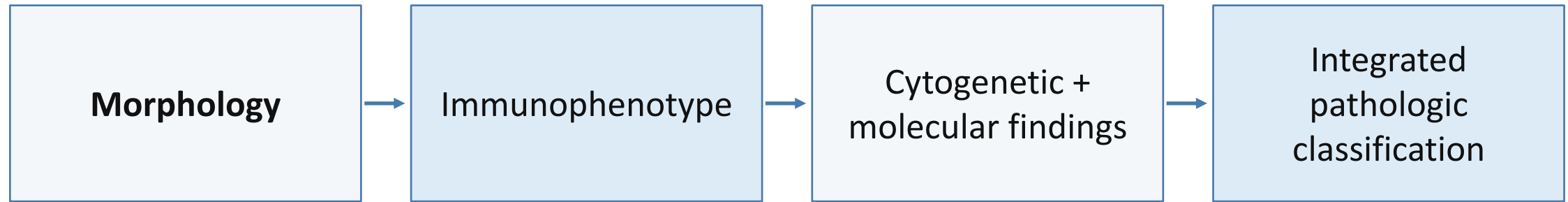
## Targeted tumor panels prioritize interpretable genes

- Targeted NGS commonly examines exons from selected cancer-associated genes.
- Focused panels concentrate sequencing resources on clinically relevant loci.
- Alterations may support diagnosis, prognosis, or selection of therapy.
- Each molecular finding still requires disease-specific interpretation.

## Hematologic neoplasms increasingly have molecular categories

- Acute leukemias and some myeloid neoplasms require detailed genetic assessment.
- Targeted sequencing can contribute to placement in molecularly defined groups.
- Cytogenetic lesions and sequence variants can complement the morphologic diagnosis.
- A molecular category must be assigned using the applicable disease framework.

# Molecular evidence must be integrated—not isolated



Clinical context, sample quality and the applicable entity definition govern the final interpretation.

# Diagnostic, prognostic and predictive biomarkers

| Use               | Question                               | Illustration                               |
|-------------------|----------------------------------------|--------------------------------------------|
| <b>Diagnostic</b> | What neoplasm is this?                 | Ewing-associated translocation             |
| <b>Prognostic</b> | What clinical behavior is anticipated? | MYCN amplification in neuroblastoma        |
| <b>Predictive</b> | Which treatment may work?              | EGFR mutation or ALK fusion in lung cancer |

## Neuroblastoma: MYCN amplification

- Amplification of the MYCN oncogene is associated with an adverse prognosis.
- Amplification creates additional copies of the oncogene in neoplastic cells.
- MYCN status contributes to neuroblastoma risk assessment.
- Amplification is a prognostic finding, not a standalone histologic diagnosis.

## Neuroblastoma: Loss of chromosome 1p

- Chromosome 1p deletion is another unfavorable genomic feature.
- Loss of a chromosomal region represents a copy-number alteration.
- Its prognostic implication differs from the molecular detection method.
- Risk assessment draws on multiple tumor and patient characteristics.

## Oligodendroglioma: Combined chromosome-arm losses

- Loss of chromosome arms 1p and 19q is an important tumor-associated alteration.
- The source associates 1p/19q loss with improved therapeutic response and survival.
- The clinical implication is distinct from those of EGFR amplification in the comparison.
- Interpret this finding within the integrated glioma diagnosis.

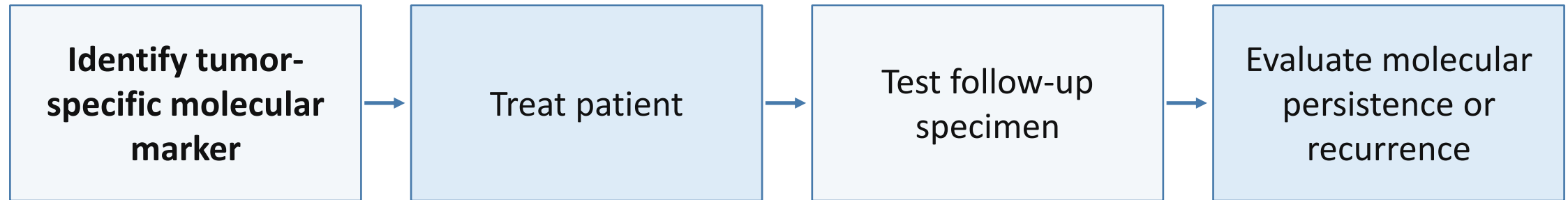
## TP53 alterations often carry adverse implications

- Mutation or deletion involving TP53 is linked to worse outcomes in many cancers.
- TP53 defects can be associated with resistance to treatment.
- The prognostic meaning varies with neoplasm type and the broader molecular context.
- Molecular results refine risk rather than fully determining an individual outcome.

## Selected prognostic alterations: Different mechanisms

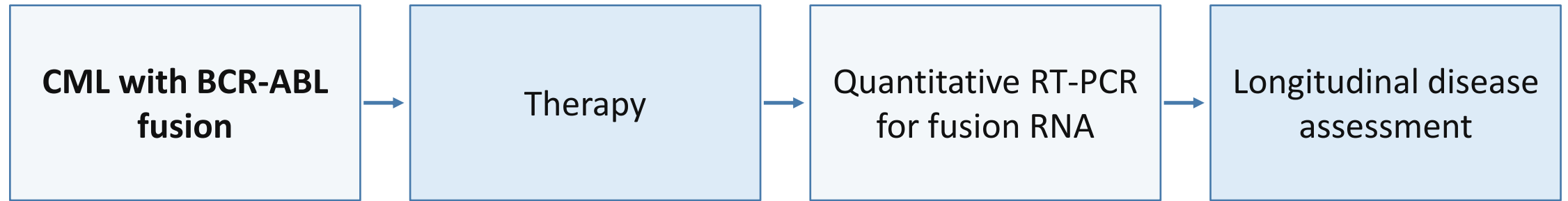
| Tumor setting           | Genetic feature           | Use emphasized in source                                  |
|-------------------------|---------------------------|-----------------------------------------------------------|
| Neuroblastoma           | MYCN amplification        | Adverse prognosis                                         |
| Neuroblastoma           | Chromosome 1p deletion    | Adverse prognosis                                         |
| Oligodendroglial tumors | 1p/19q loss               | Favorable response / longer survival in source comparison |
| Various cancers         | TP53 mutation or deletion | Often unfavorable prognosis                               |

# Molecular monitoring of minimal residual disease



The marker must be detectable with an appropriate, sufficiently sensitive method.

# CML: BCR-ABL transcript monitoring



RT-PCR can measure residual leukemia-associated transcript burden over time.

# Baseline diagnosis and post-treatment monitoring

| At diagnosis                                       | During follow-up                                           |
|----------------------------------------------------|------------------------------------------------------------|
| Establish the biologic identity of the tumor       | Track a known tumor-specific genetic signature             |
| Integrate molecular finding with initial pathology | Use serial molecular measurements to assess disease burden |
| Question: Which entity or target?                  | Question: Residual disease or emerging relapse?            |

# Somatic tumor testing and hereditary testing

| <b>Tumor (somatic) testing</b>               | <b>Constitutional (germline) testing</b>               |
|----------------------------------------------|--------------------------------------------------------|
| Asks which alterations exist in the neoplasm | Asks whether a predisposition allele is inherited      |
| May select targeted cancer therapy           | May affect screening, prevention and family counseling |
| Interprets tumor-specific molecular biology  | Has implications beyond the tested individual          |

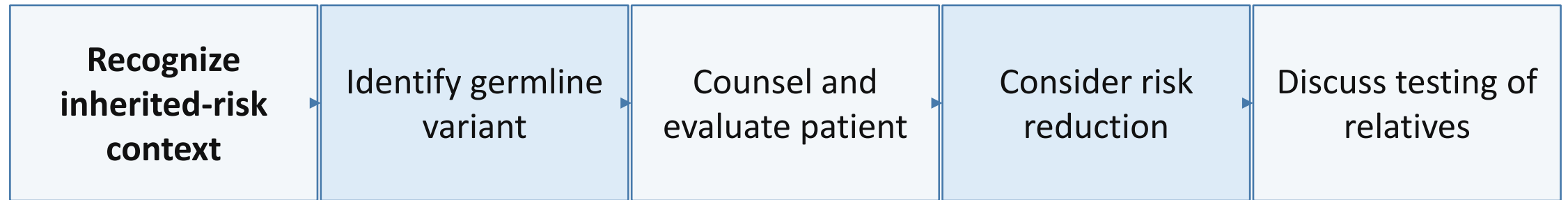
## BRCA1 and BRCA2: Hereditary susceptibility

- Pathogenic germline variants in BRCA1 and BRCA2 predispose to particular cancers.
- Testing may identify individuals requiring specialized risk assessment.
- Findings can inform surveillance or discussion of preventive interventions.
- At-risk relatives may also need appropriately structured genetic counseling.

## RET: A proto-oncogene in inherited cancer risk

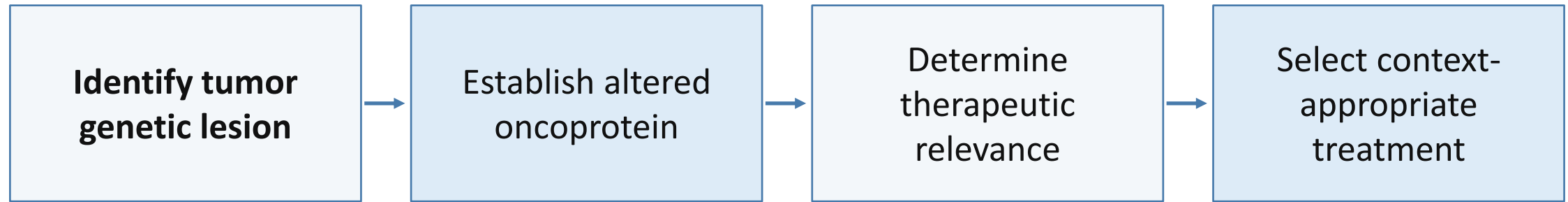
- Some inherited mutations in the RET proto-oncogene increase risk of specific neoplasms.
- Detection may enable evaluation before symptomatic cancer develops.
- A family-oriented management strategy can include targeted screening.
- Clinical decisions require variant-specific interpretation and counseling.

# Hereditary predisposition testing has consequences beyond diagnosis



Presymptomatic testing has important ethical and familial implications.

# Predictive testing connects a lesion to a drug target



A predictive molecular marker directs a treatment decision only in the relevant cancer setting.

## PML-RARA in acute promyelocytic leukemia

- The PML-RARA fusion identifies a molecular lesion relevant to treatment selection.
- Its demonstration can be urgent when acute promyelocytic leukemia is suspected.
- A specific retinoic acid receptor-associated rearrangement underlies drug sensitivity.
- Cytogenetic/FISH or molecular assays contribute to targeted management.

## BCR-ABL: Both therapeutic target and monitoring marker

- BCR-ABL is a characteristic fusion in chronic myeloid leukemia.
- It also occurs in a subset of acute lymphoblastic leukemia.
- Detection identifies an oncogenic lesion relevant to targeted treatment.
- Fusion-transcript measurement provides a separate longitudinal monitoring function.

## EGFR mutations in lung carcinoma

- Selected lung cancers contain activating mutations involving EGFR (ERBB1).
- The mutation can identify a therapeutically targetable abnormality.
- The marker is predictive in a defined clinical and tumor context.
- Subsequent molecular testing may identify resistance-associated variants.

## ALK rearrangement in lung carcinoma

- A subset of lung cancers expresses abnormal ALK fusion oncoproteins.
- Molecular testing can detect ALK gene rearrangements.
- ALK protein immunohistochemistry can provide complementary evidence.
- The result can identify tumors potentially responsive to ALK inhibitors.

## BRAF alterations in melanoma

- Some melanomas harbor activating BRAF mutations.
- The mutation can identify a candidate for an oncoprotein-directed therapy.
- A molecularly similar lesion does not guarantee equal therapeutic effect in every histologic type.
- The pathology diagnosis remains important to treatment interpretation.

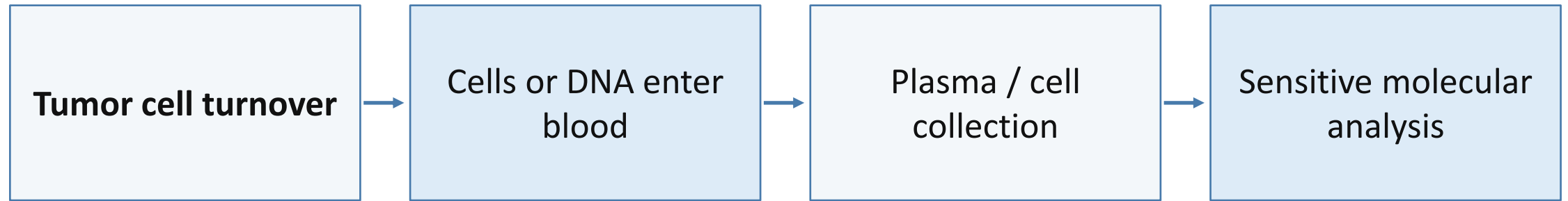
## ERBB2 / HER2: Molecular and protein-level assays

| Immunohistochemistry                               | FISH                                                  |
|----------------------------------------------------|-------------------------------------------------------|
| Detects HER2 protein overexpression in tumor cells | Assesses ERBB2 gene amplification                     |
| Measures a phenotypic oncoprotein product          | Measures the corresponding genomic copy-number lesion |
| Can guide therapy in relevant breast tumors        | May complement or clarify tissue protein testing      |

## Same pathway lesion, different histologic setting

| Tumor               | BRAF-associated treatment pattern             |
|---------------------|-----------------------------------------------|
| Hairy cell leukemia | Often sustained responses                     |
| Melanoma            | Responses may be transient                    |
| Colon carcinoma     | Limited responses in the described comparison |

# Liquid biopsy: Tumor material enters circulation

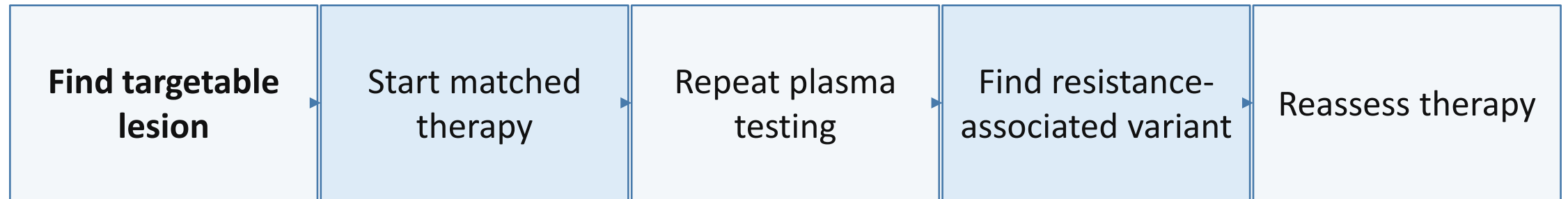


Cell-free tumor DNA derives in part from dying cancer cells; Detection depends on tumor shedding.

# Circulating tumor cells versus circulating tumor DNA

| Circulating tumor cells                 | Circulating tumor DNA                                   |
|-----------------------------------------|---------------------------------------------------------|
| Intact tumor-derived cells in blood     | Tumor-derived fragments within cell-free DNA            |
| May provide cellular material for study | Can be tested for selected driver or acquired mutations |
| Requires cell-based recovery            | Requires highly sensitive nucleic acid detection        |

# Serial ctDNA can reveal emergent resistance



In some settings, a resistance variant can be detected before clinically apparent relapse.

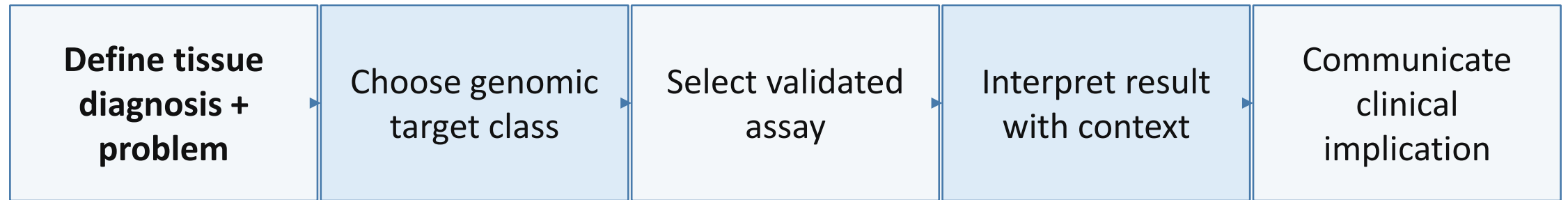
## Tissue biopsy and liquid biopsy are complementary

| Tumor tissue                                 | Plasma-based molecular test                            |
|----------------------------------------------|--------------------------------------------------------|
| Shows architecture and morphologic phenotype | Permits repeat, minimally invasive sampling            |
| Documents invasion and tumor–stroma context  | May reflect tumor-derived variants released into blood |
| Requires tissue collection                   | Does not by itself reproduce tissue histopathology     |

## Important interpretive constraints

- A negative targeted assay does not examine every possible lesion.
- Selected methods require an appropriate target or prior knowledge of a locus.
- Tissue processing and analyte preservation influence what can be measured.
- Technical findings must be reconciled with morphologic and clinical evidence.

# A clinically useful integrated testing pathway



The molecular report should distinguish diagnostic, prognostic, predictive and monitoring uses.

## Case 1 · Lymphoid proliferation

### Hypothetical vignette

A lymph-node biopsy shows a lymphoid proliferation whose reactive or neoplastic nature remains uncertain.

**Which molecular feature may help establish whether a dominant lymphocyte clone is present?**

*Consider antigen-receptor gene rearrangements.*

## Case 1 · Interpretation

- PCR can evaluate immunoglobulin or T-cell receptor rearrangements.
- A dominant rearrangement supports clonality of the relevant lymphocyte population.
- A heterogeneous rearrangement pattern supports polyclonality.
- The result must be integrated with morphology and immunophenotype.

## Case 2 · Childhood small round cell tumor

### Hypothetical vignette

Biopsy of a childhood soft-tissue mass shows a malignant small round cell tumor. Morphology is not sufficiently specific.

**What characteristic rearrangement can support Ewing sarcoma?**

*Select a fusion-associated cytogenetic target.*

## Case 2 · Interpretation

- The t(11;22)(q24;q12) rearrangement is a characteristic diagnostic clue.
- PCR-based molecular detection can help confirm Ewing sarcoma.
- FISH or an appropriate transcript assay can provide complementary testing pathways.
- The molecular finding supports—not replaces—the overall tumor diagnosis.

## Case 3 · Treated CML

### Hypothetical vignette

A patient with known chronic myeloid leukemia is receiving treatment. The question is whether molecular disease persists.

**Which analyte should be followed serially?**

*The tumor has a characteristic expressed fusion gene.*

## Case 3 · Interpretation

- BCR-ABL fusion RNA can be quantified using a reverse-transcription PCR approach.
- Serial assays evaluate molecular disease burden rather than initial tumor morphology.
- A changing transcript measurement may indicate residual or recurrent disease.
- Interpretation depends on assay and clinical follow-up context.

## Case 4 · Drug resistance in lung cancer

### Hypothetical vignette

A patient with molecularly targeted treatment for lung carcinoma develops concern for emerging resistance. Repeated tissue biopsy is undesirable.

**How can the tumor genotype be reevaluated from blood?**

*The blood may contain DNA shed by neoplastic cells.*

## Case 4 · Interpretation

- A plasma-based liquid biopsy can interrogate circulating tumor DNA.
- Sensitive molecular analysis can seek a resistance-associated sequence variant.
- Repeated sampling can potentially detect genomic evolution before overt relapse.
- Tissue remains important when morphology or broader evaluation is needed.

## One clinical question → one molecular strategy

| Clinical question                        | Informative target         | Representative strategy       |
|------------------------------------------|----------------------------|-------------------------------|
| Is the infiltrate clonal?                | IG / TCR rearrangement     | PCR                           |
| Does the tumor harbor a hallmark fusion? | Chromosomal / RNA fusion   | FISH, cytogenetics, RT-PCR    |
| What is the molecular category?          | Selected sequence variants | Targeted NGS                  |
| Is disease still present?                | Clone-specific transcript  | Quantitative RT-PCR           |
| Is treatment resistance evolving?        | New tumor-derived variant  | Sensitive liquid-biopsy assay |

# Core synthesis

- Match the molecular technique to the alteration and the clinical decision.
- Clonality, translocation, copy-number and sequence assays answer different questions.
- The same test result can serve diagnostic, prognostic or predictive roles depending on context.
- Molecular data increase—not eliminate—the need for histopathologic interpretation.

# Primary Robbins source and supporting methods

**Robbins, Cotran & Kumar. Pathologic Basis of Disease. 11th ed. (2026).**

Primary section: Neoplasia — Molecular Diagnostics and Cytogenetics.

Technical methods: Genes and Human Diseases — Molecular Genetic Diagnosis.

Adjacent topics consulted: Immunohistochemistry, Molecular Profiling of Tumors and Neuroblastoma.

All schematics in this lecture are original editable teaching diagrams, not clinical images.