Tumor Profiling: Comprehensive Guide

NGS Panels

Next-generation sequencing for comprehensive mutation analysis

TMB Assessment

Tumor Mutational Burden predicts immunotherapy response

MSI Status

Microsatellite Instability as biomarker for treatment selection

HRD Testing

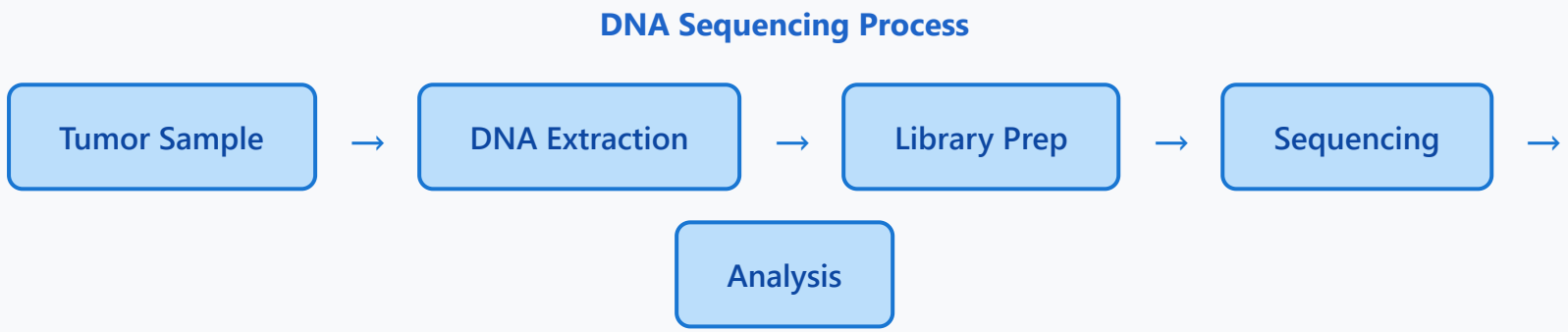
Homologous Recombination Deficiency guides PARP inhibitor use

PD-L1 Expression: Key biomarker for immune checkpoint inhibitor therapy eligibility

Detailed Analysis of Tumor Profiling Methods

▶ 1. NGS Panels (Next-Generation Sequencing)

Next-generation sequencing panels enable simultaneous analysis of multiple genes, providing comprehensive genomic profiling of tumors. This technology has revolutionized precision oncology by identifying actionable mutations that guide targeted therapy selection.



Example: Point Mutation Detection

`ATGC GCTA ATGC` → `ATGC GTTA ATGC`

Normal DNA → Mutated DNA (C→T mutation)

Key Features:

- **Multi-gene analysis:** Simultaneous testing of 50-500+ genes
- **Mutation types detected:** SNVs, indels, CNVs, fusions
- **Applications:** Targeted therapy selection, clinical trial matching
- **Turnaround time:** Typically 7-14 days

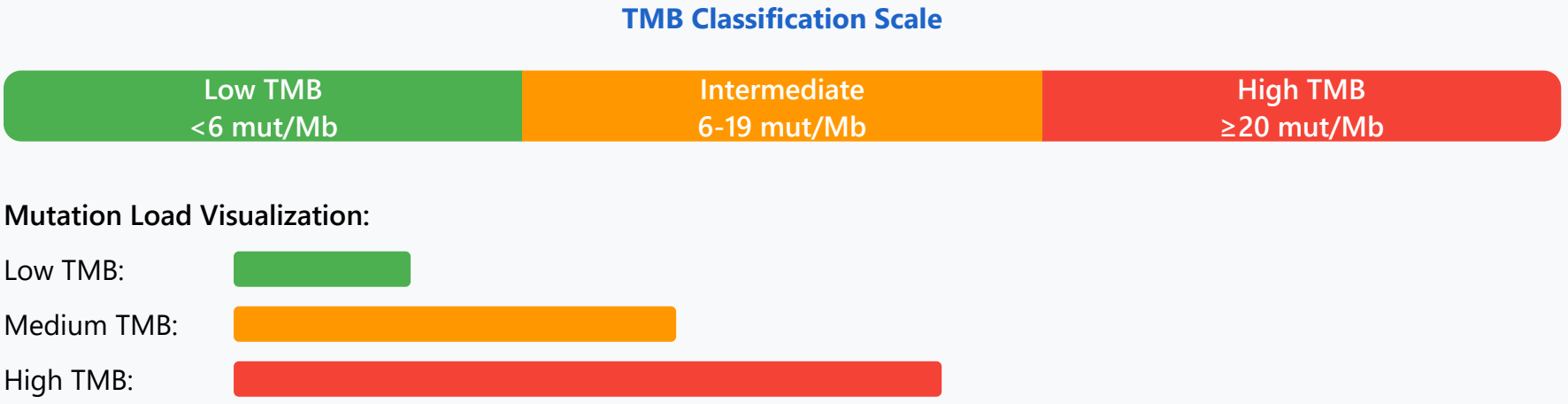
- **Sample requirements:** FFPE tissue or liquid biopsy (ctDNA)

Clinical Significance:

NGS panels identify actionable mutations in genes such as EGFR, ALK, BRAF, KRAS, and HER2, enabling precision medicine approaches. For example, EGFR mutations in lung cancer predict response to tyrosine kinase inhibitors like erlotinib and osimertinib.

▶ 2. TMB Assessment (Tumor Mutational Burden)

Tumor Mutational Burden quantifies the total number of somatic mutations per megabase of DNA. High TMB tumors generate more neoantigens, making them more immunogenic and likely to respond to immune checkpoint inhibitors.



Key Features:

- **Measurement:** Number of mutations per megabase (mut/Mb)
- **Cutoff values:** TMB-High typically defined as ≥10-20 mut/Mb
- **Cancer types with high TMB:** Melanoma, NSCLC, bladder cancer, MSI-H tumors
- **Mechanism:** More mutations → more neoantigens → enhanced immune recognition
- **FDA approval:** Pembrolizumab approved for TMB-H tumors (≥10 mut/Mb)

Clinical Significance:

High TMB predicts improved response to immune checkpoint inhibitors across multiple cancer types. Studies show that patients with TMB-H tumors have higher response rates (29-50%) to immunotherapy compared to TMB-low tumors (10-20%). However, TMB should be interpreted in context with other biomarkers like PD-L1 expression and MSI status.

▶ 3. MSI Status (Microsatellite Instability)

Microsatellites are repetitive DNA sequences that are prone to replication errors. MSI occurs when mismatch repair (MMR) genes are deficient, leading to accumulation of mutations in these regions. MSI-High tumors are highly responsive to immunotherapy.

MSI Comparison

MSI-Stable (MSS)	MSI-High

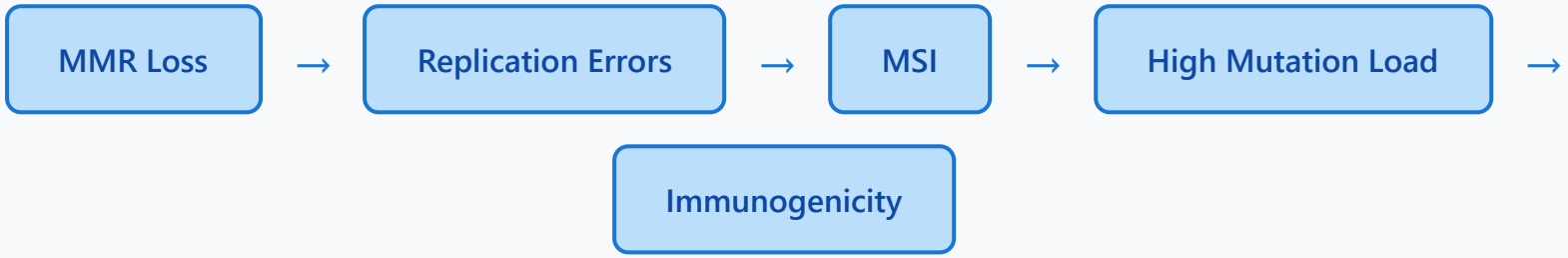
Normal: $(CA)_{15}$
Tumor: $(CA)_{15}$
No length change

Intact MMR system

Normal: $(CA)_{15}$
Tumor: $(CA)_{22}$
Length variation

Deficient MMR (dMMR)

MMR Gene Deficiency Cascade:



Key Features:

- **Classification:** MSI-High (MSI-H), MSI-Low (MSI-L), Microsatellite Stable (MSS)
- **MMR genes:** MLH1, MSH2, MSH6, PMS2
- **Detection methods:** PCR-based assay, NGS, IHC for MMR proteins
- **Common cancers:** Colorectal (15%), endometrial (30%), gastric (20%)
- **Lynch syndrome:** Hereditary MSI-H due to germline MMR mutations

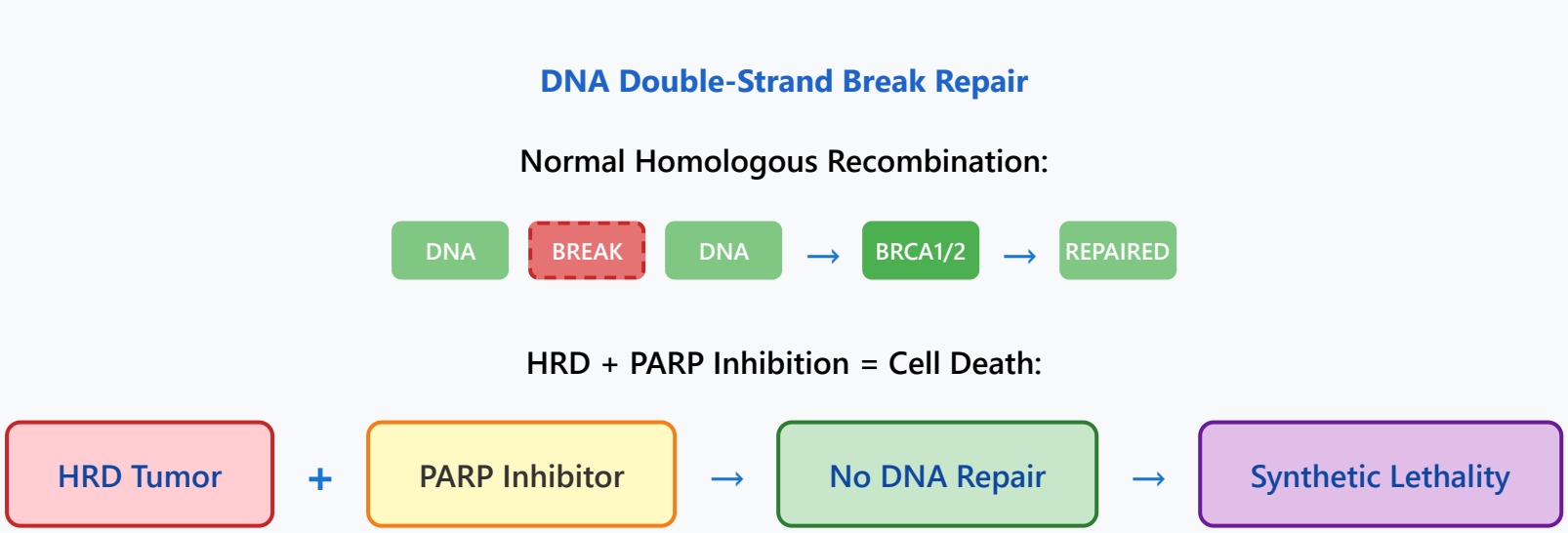
Clinical Significance:

MSI-H/dMMR tumors are highly responsive to immune checkpoint inhibitors regardless of tumor type. FDA has granted tissue-agnostic approvals for pembrolizumab and nivolumab in MSI-H cancers. Response rates exceed 50% in MSI-H tumors

compared to <10% in MSS tumors. MSI-H colorectal cancer also has better prognosis and may not benefit from 5-FU chemotherapy.

▶ 4. HRD Testing (Homologous Recombination Deficiency)

Homologous Recombination is a crucial DNA repair pathway for double-strand breaks. Deficiency in this pathway (HRD) makes cancer cells vulnerable to PARP inhibitors, which block an alternative DNA repair mechanism, leading to synthetic lethality.



Key Features:

- **Key genes:** BRCA1, BRCA2, RAD51, PALB2, ATM, CHEK2
- **HRD score:** Composite measure of genomic instability (LOH, TAI, LST)

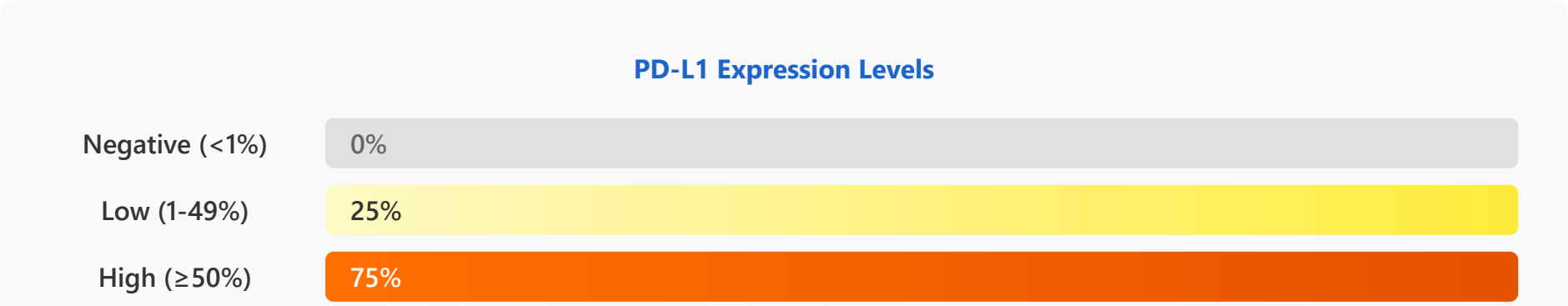
- **Testing methods:** Germline/somatic sequencing, HRD genomic scar analysis
- **Primary cancers:** Ovarian (50%), breast (20%), prostate, pancreatic
- **PARP inhibitors:** Olaparib, rucaparib, niraparib, talazoparib

Clinical Significance:

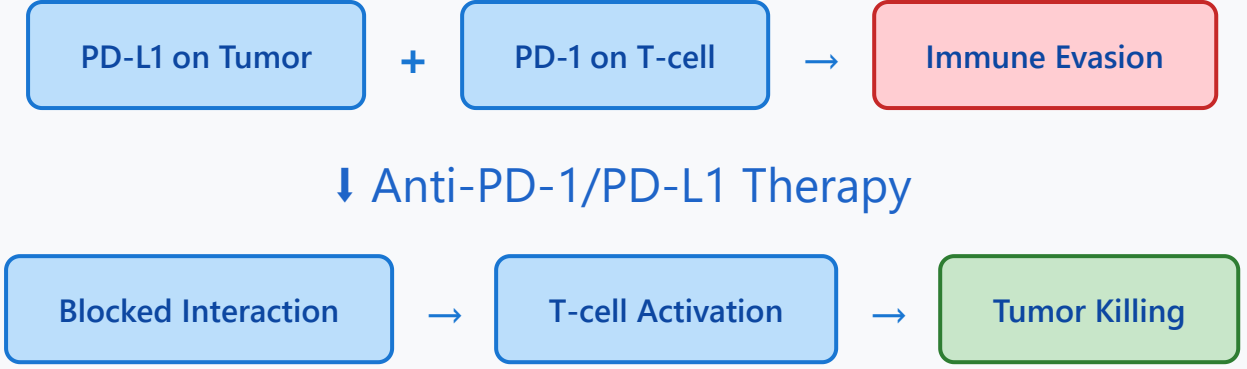
HRD testing identifies patients who will benefit from PARP inhibitors. FDA-approved indications include BRCA-mutated ovarian, breast, pancreatic, and prostate cancers. HRD-positive tumors show response rates of 50-80% to PARP inhibitors with significant progression-free survival benefits. Beyond BRCA mutations, broader HRD testing captures additional patients with genomic scars who may respond to treatment.

▶ 5. PD-L1 Expression Analysis

PD-L1 (Programmed Death-Ligand 1) is a protein expressed on tumor and immune cells that suppresses T-cell activation. PD-L1 expression levels help predict response to immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway.



Checkpoint Inhibitor Mechanism:



Key Features:

- **Testing method:** Immunohistochemistry (IHC) on tumor tissue
- **Scoring systems:** TPS (Tumor Proportion Score), CPS (Combined Positive Score)
- **Cutoffs vary by cancer:** NSCLC (≥50% for first-line), head/neck (≥1%)
- **Checkpoint inhibitors:** Pembrolizumab, nivolumab, atezolizumab, durvalumab
- **Limitations:** Dynamic expression, intratumoral heterogeneity, assay variability

Clinical Significance:

PD-L1 expression is a companion or complementary diagnostic for multiple checkpoint inhibitors. In NSCLC, PD-L1 ≥50% predicts superior outcomes with pembrolizumab monotherapy versus chemotherapy. However, PD-L1 is not a perfect biomarker—responses can occur in PD-L1 negative tumors, and some PD-L1 positive tumors don't respond. Integration with TMB and MSI status improves patient selection.

Integrated Biomarker Approach

Modern precision oncology relies on comprehensive tumor profiling combining NGS panels, TMB, MSI, HRD, and PD-L1 testing. This integrated approach maximizes therapeutic opportunities by identifying patients for targeted therapies, immunotherapy, and DNA damage repair inhibitors. Multi-biomarker testing ensures optimal treatment selection and improves patient outcomes across diverse cancer types.