Circulating Tumor DNA as a Marker for Minimal Residual Disease in Pediatric Rhabdomyosarcoma

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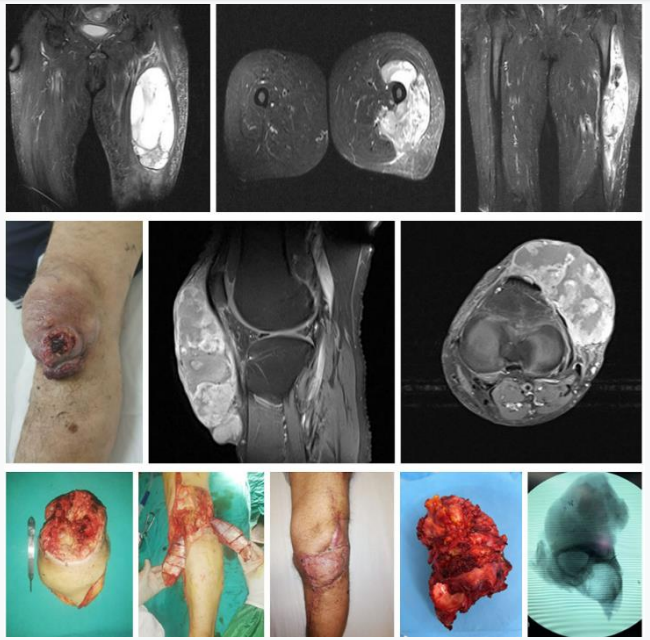
Presentation Outline

1. Introduction to **Rhabdomyosarcoma**
2. Overview of RMS: Epidemiology & Subtypes
3. Understanding **Minimal Residual Disease**
4. Circulating Tumor DNA: Biological Basis
5. Role of ctDNA in RMS
6. Clinical Applications in Pediatric Oncology
7. Challenges and Limitations
8. Future Directions in ctDNA Research
9. Case Studies and Clinical Trials
10. Comparison with Other Biomarkers & Conclusion

Introduction to Rhabdomyosarcoma



What is Rhabdomyosarcoma?

Malignant tumors arising from **mesenchymal cells** with skeletal muscle differentiation

Most common **soft tissue sarcoma** in children and adolescents

50%

Of sarcomas in children

Accounts for nearly half of all pediatric sarcomas

Primarily affects **children** and adolescents

More than 50% in patients up to 20 years old

Variable **prognosis**

5-year survival: 70-90% for localized, <30% for metastatic

Novel biomarkers needed for **risk stratification**

MRD detection and treatment monitoring crucial

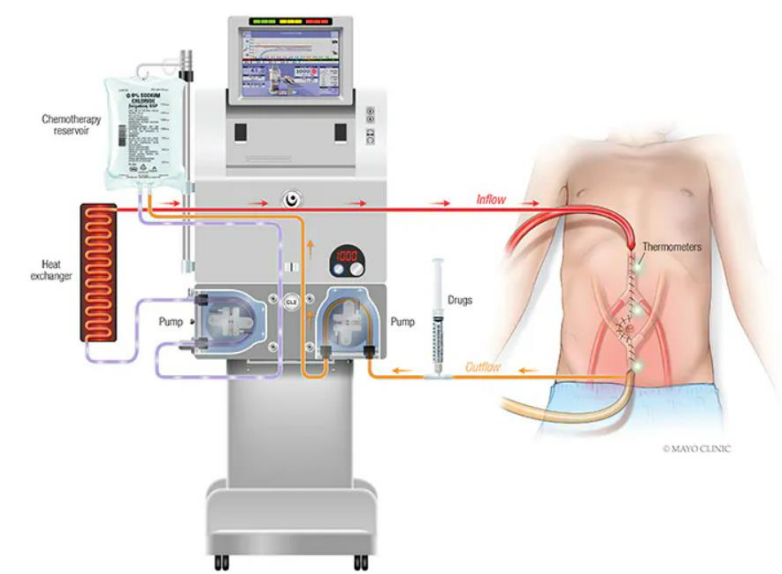
Current Challenges in RMS Management

Despite advances in multimodal therapy...

Outcomes remain **suboptimal** for high-risk patients

Identification of **novel biomarkers** is crucial for:

- Risk stratification
- Minimal residual disease (MRD) detection
- Treatment monitoring



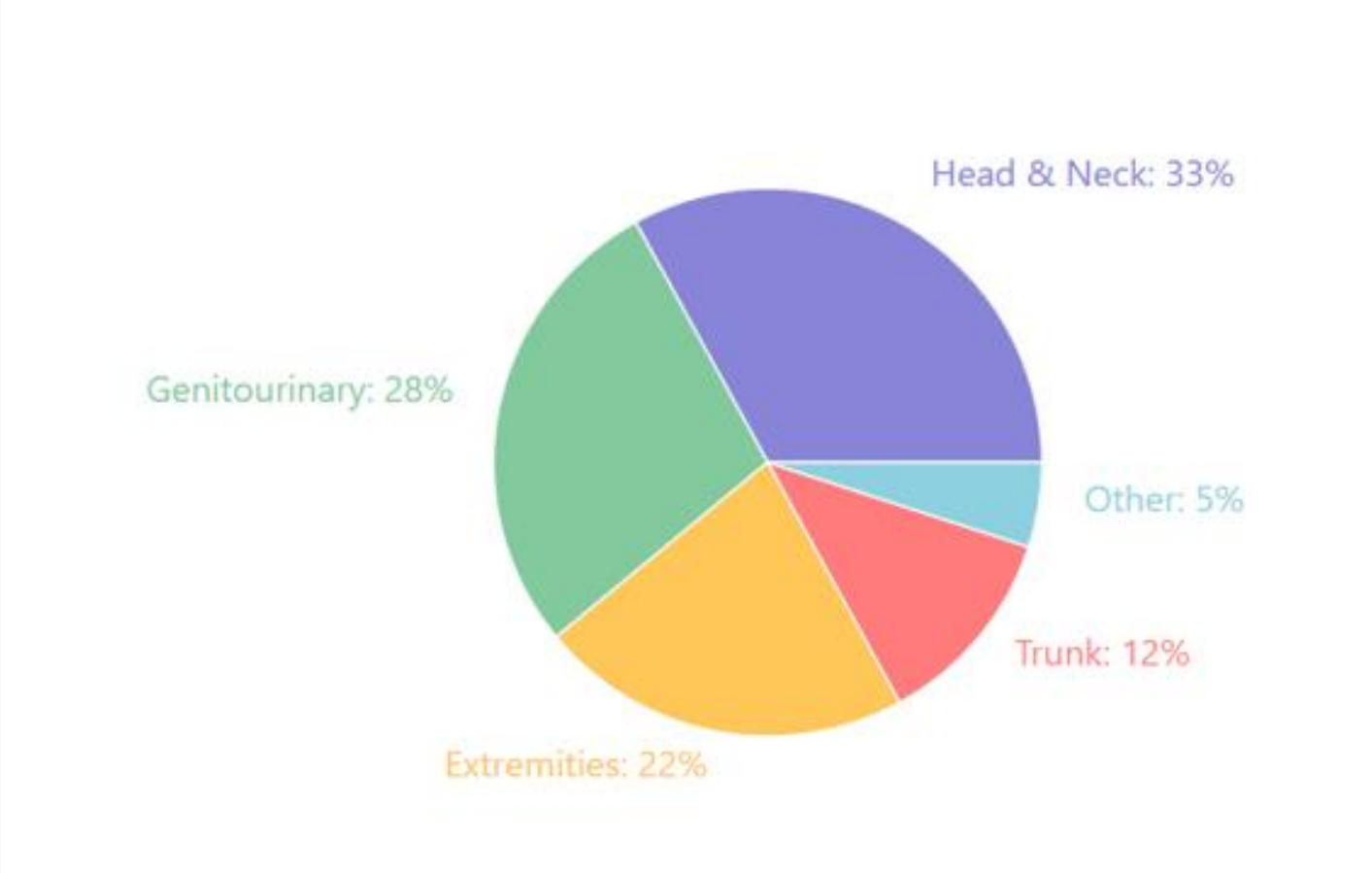
Poor survival in metastatic disease
Less than 30% 5-year survival rate

Relapse after initial treatment
20% of localized disease patients relapse

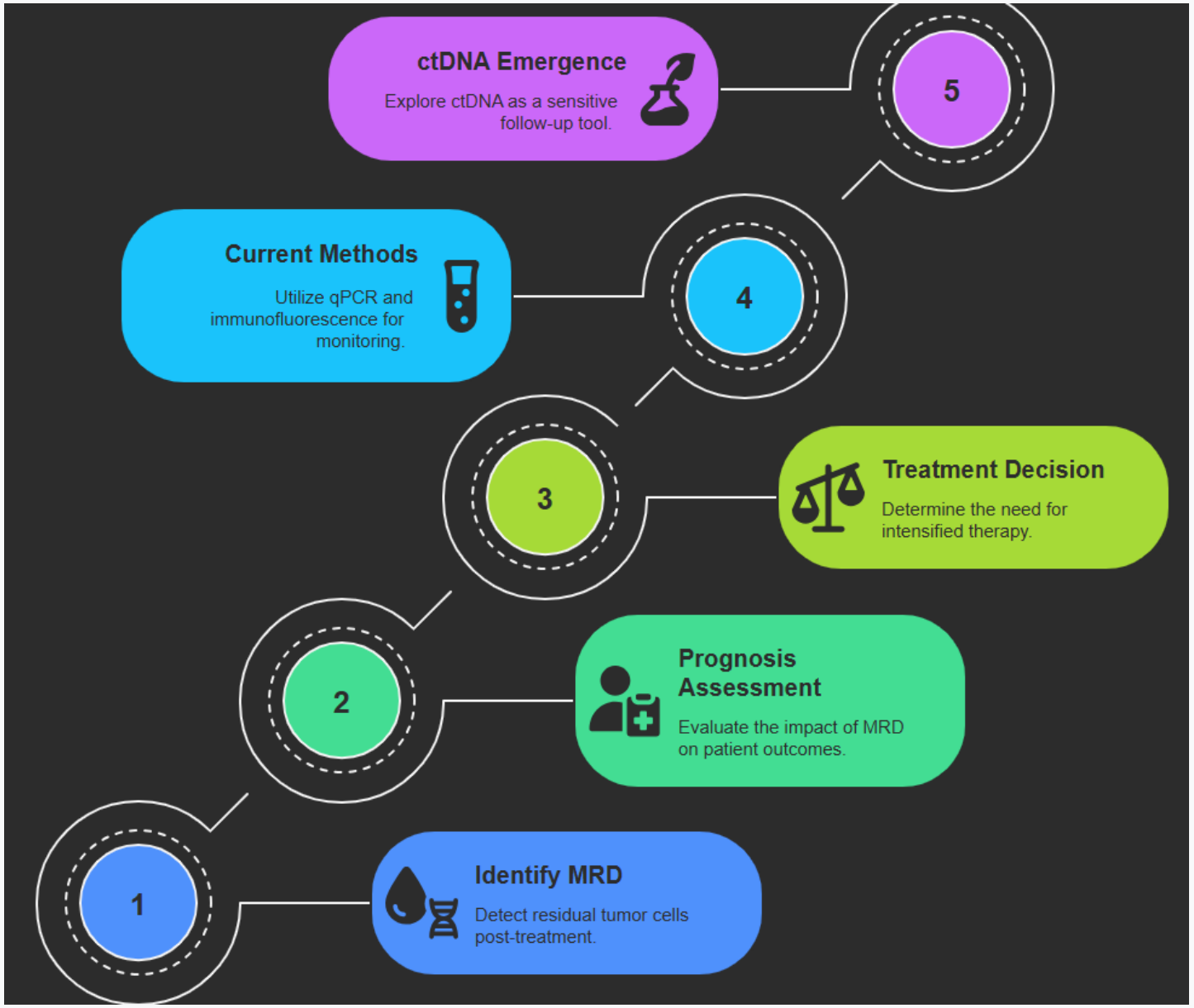
Limited biomarkers for early detection
Current methods lack sensitivity and specificity

Invasive procedures for monitoring
Bone marrow sampling requires sedation in children

Overview of Rhabdomyosarcoma - Epidemiology



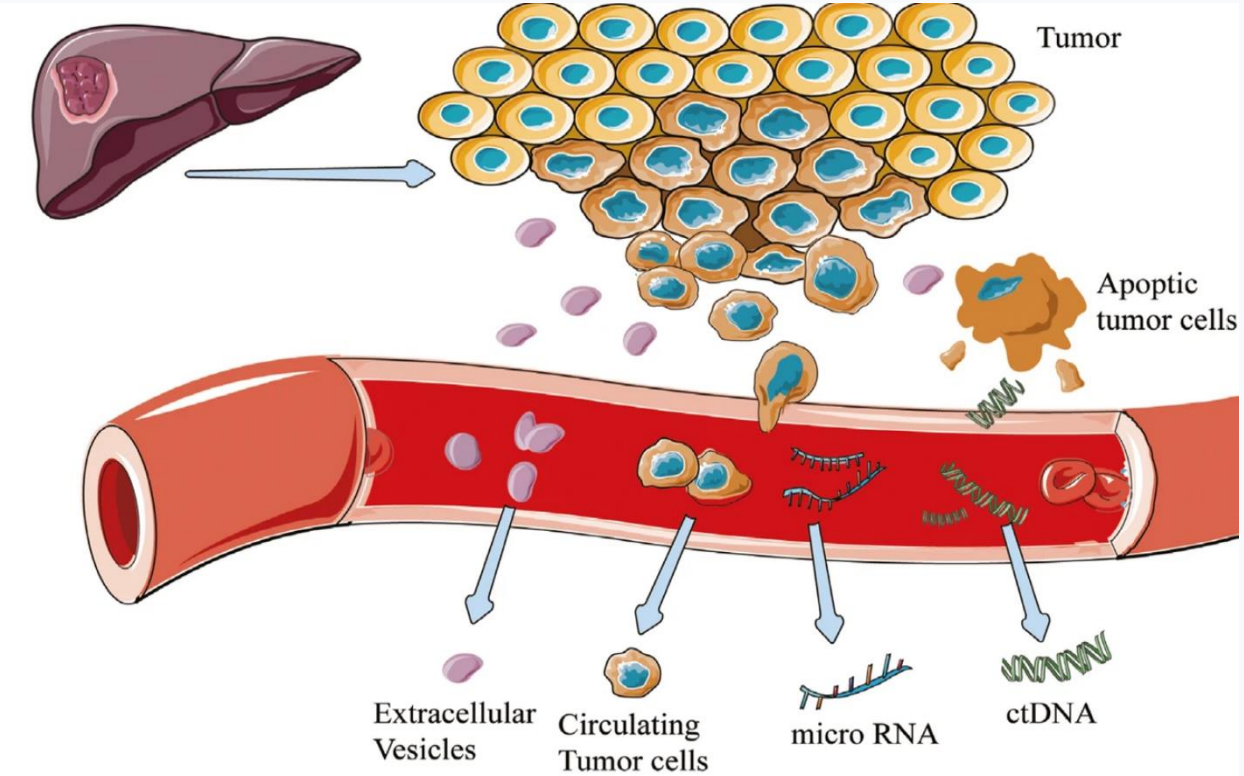
Understanding Minimal Residual Disease



Current Methods of MRD Detection

Method	Sensitivity	Specificity	Invasiveness	Main Limitations	Cost
qPCR	High (10^{-4}-10^{-6})	High	Moderate (bone marrow)	Not universally applicable, requires specific targets	Moderate
Flow Cytometry	Moderate (10^{-3}-10^{-4})	Moderate	Moderate (bone marrow)	Limited prognostic validation	High
Immunocytology	Low-Moderate (10^{-3})	Variable	Low (blood sample)	Variable protein expression, low reproducibility	Low
ctDNA	High (10^{-4}-10^{-5})	High	Very Low (blood sample)	Technology still developing, standardization needed	High (currently)

Circulating Tumor DNA - Biological Basis



Advantages Over Tissue Biopsy

- **Minimally invasive** (liquid biopsy)
- Enables **longitudinal monitoring**
- Captures **tumor heterogeneity**
- Detects **micrometastases** and MRD

Circulating Tumor DNA - Methods of Analysis

1

Sample Collection

Blood draw in specialized tubes

2

cfDNA Enrichment

Magnetic beads or polyethylenglycol

3

DNA Extraction

Isolation of cfDNA from plasma

4

Analysis

Detection of tumor-specific alterations

Sequencing Approaches

- **Whole-genome sequencing** (WGS)
- **Whole-exome sequencing** (WES)
- **Targeted sequencing** (big panels)
- **Shallow WGS** (sWGS)

Detectable Alterations

- **Point mutations**
- **Copy number changes**
- **Insertions/deletions**
- **Gene rearrangements**
- **DNA methylation** patterns

Technical Considerations

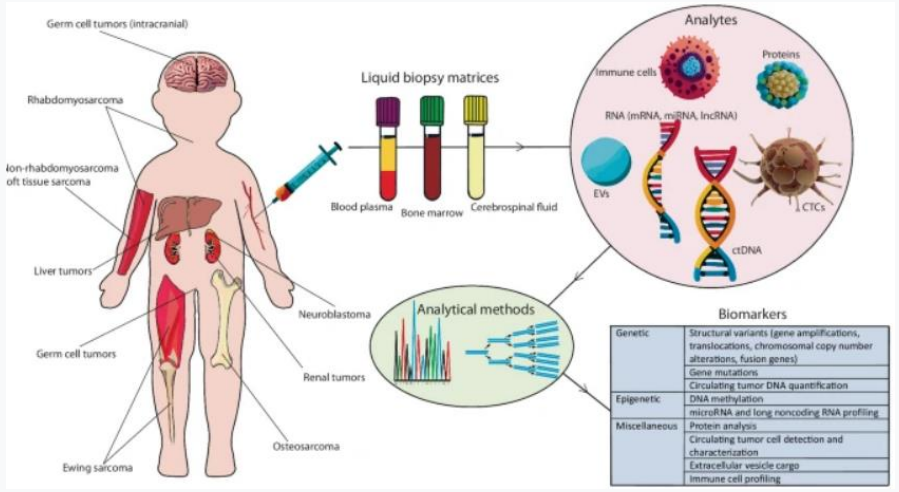
- **Short half-life** allows recent monitoring
- **Prior knowledge** needed for mutation-driven analysis
- **Phosphorylation-based** protocols for rare diseases
- **High sensitivity** required for MRD

Role of ctDNA in Rhabdomyosarcoma - ctDNA as a Biomarker for MRD

ctDNA as MRD Marker

- **Persistent detection** of ctDNA after treatment correlates with relapse risk
- **Normalizing levels** indicate favorable prognosis
- Enables **real-time monitoring** of tumor burden
- **Complementary** to circulating tumor cell (CTC) evaluation
- **Detectable** in 60-70% of patients at diagnosis

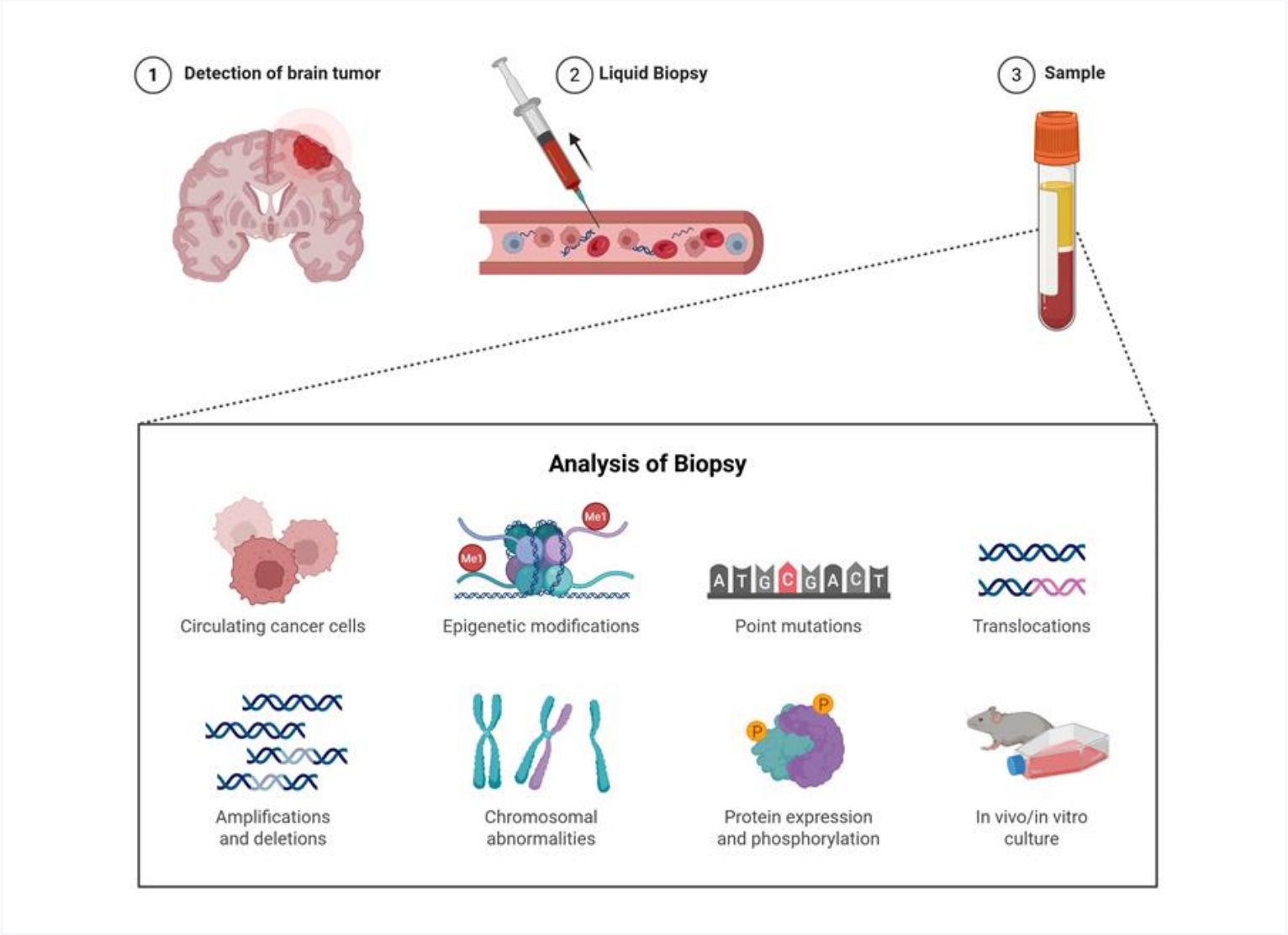
Clinical Utility in RMS



- **Localized RMS** and local relapse produce limited CTC numbers
- **Metastatic cases** show higher levels of ctDNA and CTCs
- **Bone marrow analysis** reveals disseminated tumor cells in all cases

60-70%

Role of ctDNA in Rhabdomyosarcoma - Prognostic Implications



Role of ctDNA in Rhabdomyosarcoma - Relationship with CTCs and DTCs

Biomarker Comparison

CTCs

Low numbers in localized disease

Elevated levels in metastasis

Detected at diagnosis in patients who later relapse

ctDNA

Complements CTC evaluation

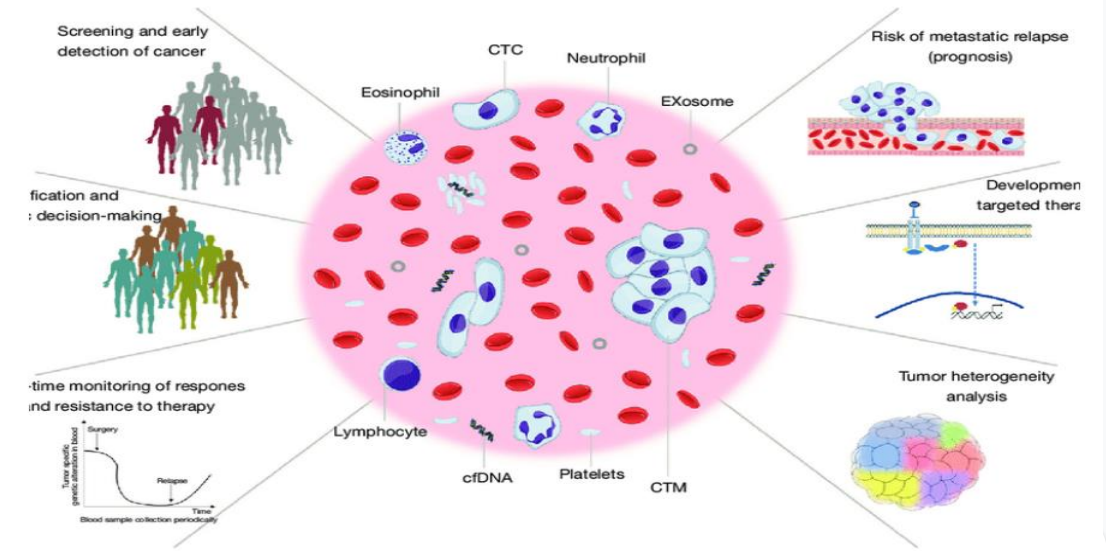
Provides **molecular profile** of tumor

Enables longitudinal monitoring of treatment response

Disseminated Tumor Cells (DTCs)

- Detected in **bone marrow** of all investigated RMS cases
- Suggests marrow as **early dissemination site**
- DTC amplification associated with **adverse survival**

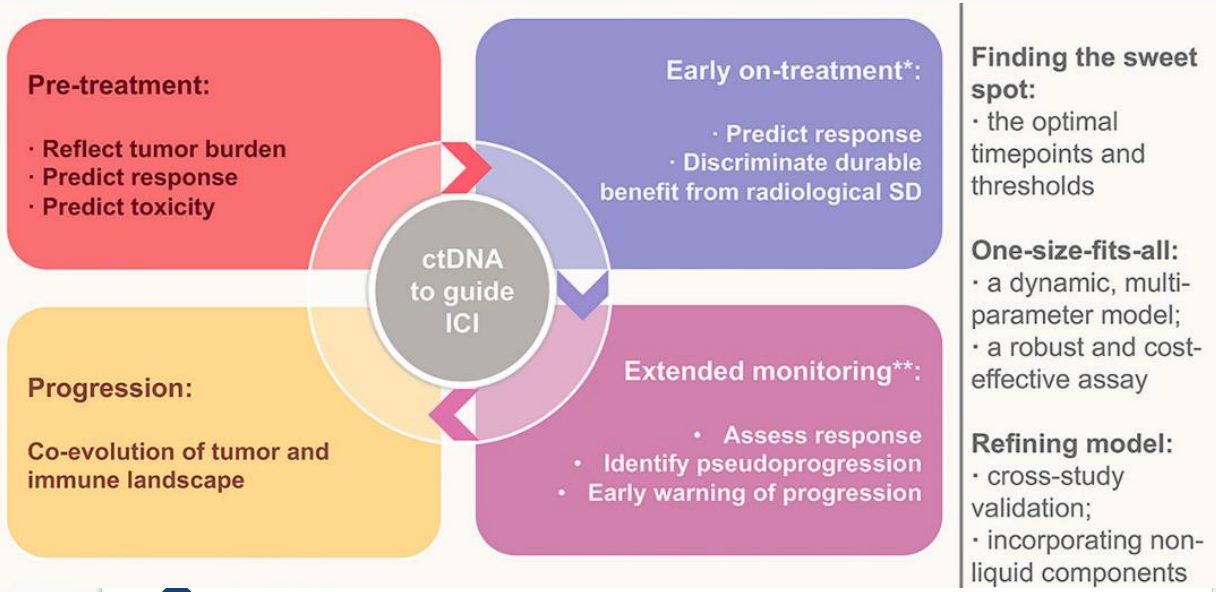
Molecular Characterization



- **Genotype concordance** among CTCs, cfDNA, and primary tumor cells
- **Integrated analyses** provide comprehensive tumor biology depiction
- **Combinatorial approaches**: PCR, flow cytometry, NGS
- Mutations prominently identified in **alveolar RMS**

Clinical Applications of ctDNA in Pediatric Oncology - Monitoring Treatment Response

Treatment Response Monitoring



- ctDNA levels **correlate with tumor burden** during therapy
- **Serial assessment** enables real-time monitoring
- **Declining levels** indicate positive treatment response
- **Persistent detection** suggests residual disease

Advantages Over Current Methods

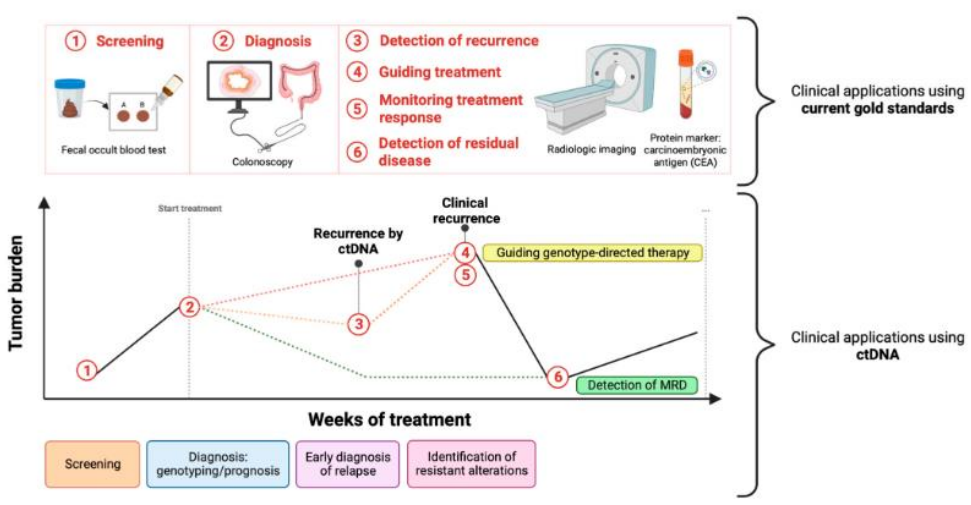
- **Minimally invasive** compared to bone marrow sampling
- Allows **frequent monitoring** without sedation
- **Faster results** than conventional imaging

Current Gold Standard	ctDNA Approach
Bone marrow sampling + flow cytometry or RQ-PCR	Plasma analysis + tumor-specific DNA detection
Limited by available cells/DNA	Higher sensitivity for MRD detection

- Enables **therapy modulation** based on molecular response

Clinical Applications of ctDNA in Pediatric Oncology - Predicting Relapse

Early Relapse Detection



ctDNA provides **lead time** of several weeks before clinical relapse

Rising levels during follow-up indicate impending progression

Sensitive detection of residual disease after treatment

One in three RMS patients experience relapse

Clinical Evidence

PAX3-FOXO1 fusion ctDNA detection correlates with poor prognosis

Droplet digital PCR detection from archival plasma samples

Post-treatment ctDNA presence associated with **tumor relapse**

Detection Method

Clinical Utility

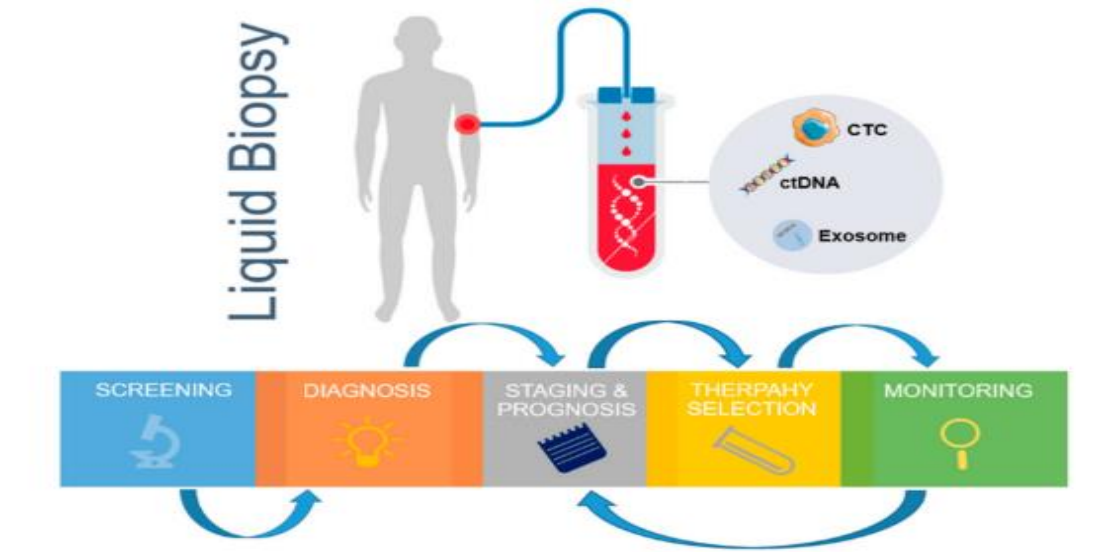
Identifies high-risk patients

Clinical Applications of ctDNA in Pediatric Oncology - Integration in Clinical Workflows

Clinical Integration Pathway

1. **Diagnosis**
   Baseline ctDNA profiling from tumor tissue
2. **Treatment Planning**
   Risk stratification based on initial ctDNA levels
3. **During Therapy**
   Serial monitoring of ctDNA dynamics
4. **Post-Treatment**
   MRD assessment to guide surveillance intensity

Clinical Benefits & Challenges



- **Personalized** treatment approaches
- **Rapid** molecular assessment
- **Reduced** invasive procedures
- **Dynamic** treatment adaptation

Challenges and Limitations - Biological Variability and Ethical Considerations

Biological Variability

Fluctuating Levels

ctDNA concentrations vary due to **tumor necrosis**, inflammation, and chemotherapy stress

Timing Issues

Post-treatment collection timing affects tumor-derived DNA amount in circulation

Tumor Burden

Low tumor burden results in limited ctDNA release, complicating detection

Multiple Measurements

Single measurement insufficient; trends from multiple samples required

Ethical Considerations

- **Sample collection challenges** in younger patients
- **No established guidelines** for sampling frequency when tumor status unknown
- **High costs** restrict widespread screening implementation
- **Psychological impact** of detecting minimal residual disease

Risk-Benefit Balance

In pediatric RMS, careful consideration of risk-benefit ratio is essential when performing genetic analyses that also profile the germline

Conclusion - Summary of Key Findings

Key Findings

Detection Rate

ctDNA detectable in **60-70%** of pediatric RMS patients at diagnosis

Prognostic Value

Absence of detectable ctDNA indicates more favorable prognosis

Treatment Monitoring

Enables **real-time tracking** of therapeutic response

Early Relapse Detection

Clinical Implications

Personalized Medicine

ctDNA analysis enables **risk stratification** and treatment adaptation

Complementary Approach

Works best when **combined** with other biomarkers and clinical assessments

Future Directions

Further studies needed to refine technologies, implement ctDNA detection in clinical workflows, and identify new opportunities to personalize pediatric RMS management

Conclusion - Future Research Directions

Research Priorities

Technology Refinement

Enhanced sensitivity for low-abundance mutations

Standardized protocols for sample processing

Larger Cohorts

Multicenter studies to validate findings

Longitudinal data across treatment phases

Multi-analyte Integration

Combined biomarkers for comprehensive monitoring

Multiomic approaches to

Novel Targets

Methylation signatures for improved specificity

RMS-specific markers for enhanced detection

Clinical Implementation

Integration into **treatment protocols** as standard of care

Development of **risk-adapted** treatment strategies

Education programs for clinicians on ctDNA interpretation

Establishment of **clinical guidelines** for ctDNA use in RMS

Vision for the Future

ctDNA analysis will become an integral component of precision medicine in pediatric oncology, enabling earlier intervention, reduced toxicity, and improved survival outcomes for children with rhabdomyosarcoma

Conclusion - Summary of Key Findings

Key Findings on ctDNA in RMS

- ctDNA serves as a **promising biomarker** for minimal residual disease detection in pediatric rhabdomyosarcoma
- Detectable in **60-70%** of patients at diagnosis, comparable to bone marrow or blood-derived tumor cells
- **Persistent detection** after treatment correlates with increased relapse risk
- **Normalizing levels** indicate favorable prognosis and treatment response
- Provides **lead time** of several weeks before clinical relapse manifestation
- Enables **real-time monitoring** of therapy response and tumor evolution

Clinical Impact

20%

of localized RMS patients relapse due to residual tumor cells undetected by conventional methods

- Potential for **personalized treatment** approaches based on MRD status
- Balancing **treatment intensity** with risk of relapse

Future Directions

Refinement of technologies and implementation in clinical workflows will enhance personalized management of pediatric RMS

Conclusion - Clinical Implications of ctDNA for RMS Management

Clinical Applications

Risk Stratification

Molecular risk assessment beyond clinical factors

Treatment Monitoring

Real-time response evaluation during therapy

Early Relapse Detection

Lead time before clinical manifestation

Personalized Therapy

Treatment adaptation based on MRD status

Minimally invasive alternative to repeated tissue biopsies and bone marrow sampling

Future Impact

Treatment de-escalation for low-risk patients with undetectable ctDNA

Treatment intensification for high-risk patients with persistent ctDNA

Novel therapeutic targets identified through molecular characterization

Dynamic treatment adaptation based on molecular response

Transformative Potential

Integration of ctDNA analysis into clinical practice has the potential to revolutionize risk stratification and treatment personalization in pediatric RMS

Future Directions in ctDNA Research - Innovative Technologies

Next-Generation Sequencing Approaches

WGS/WES

Whole-genome/exome sequencing for comprehensive genomic profiling

sWGS

Shallow whole-genome sequencing for cost-effective CNV detection

Methylation Analysis

Epigenetic profiling for tissue-of-origin identification

Targeted Sequencing

High-depth coverage of specific genomic regions

These approaches enable detection of **copy-number variations** associated with disease burden

Can identify **mutations confined to sublocalizations** within tumors

Advanced Analytical Techniques

Multi-analyte liquid biopsy combining ctDNA, CTCs, exosomes, and miRNA

Tumor heterogeneity characterization through integrated analysis

Resistance mechanisms identification in refractory/relapsed disease

Clonal evolution tracking throughout treatment course

Future Potential

Integration of multiomic data in liquid biopsy context will enable more comprehensive tumor characterization and guide personalized treatment strategies in pediatric RMS

Future Directions in ctDNA Research - Potential for Personalized Medicine

Personalized Treatment Approaches

Risk Stratification

ctDNA-based MRD assessment categorizes patients into risk groups

Treatment De-escalation

Low-risk patients may avoid excessive treatment toxicity

Treatment Intensification

High-risk patients identified for more aggressive therapy

Dynamic Adaptation

Real-time monitoring enables treatment modification

Clinical Implementation

- Several trials now incorporate ctDNA as an **outcome and stratification measure**
- Particularly valuable in **pediatric oncology** where overtreatment is a major concern
- Enables **early intervention** in patients showing molecular relapse
- Guides **targeted therapy** selection based on detected mutations

Future Vision

ctDNA analysis will likely play a growing role in risk-adapted treatment approaches, ultimately improving survival while reducing therapy-related toxicity in pediatric RMS patients

Contact Information & Questions

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Questions & Discussion

Thank you for your attention. I welcome any questions or comments about circulating tumor DNA as a marker for minimal residual disease in pediatric rhabdomyosarcoma.

Thank You

For your attention to this presentation on circulating tumor DNA as a marker for minimal residual disease in pediatric rhabdomyosarcoma

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