COLLEGE of AMERICAN PATHOLOGISTS

Emerging Concepts on Liquid Biopsy Testing

Abhijit A. Patel, MD, PhD and Pranil Chandra, DO, FCAP

June 14, 2017

Webinar Host

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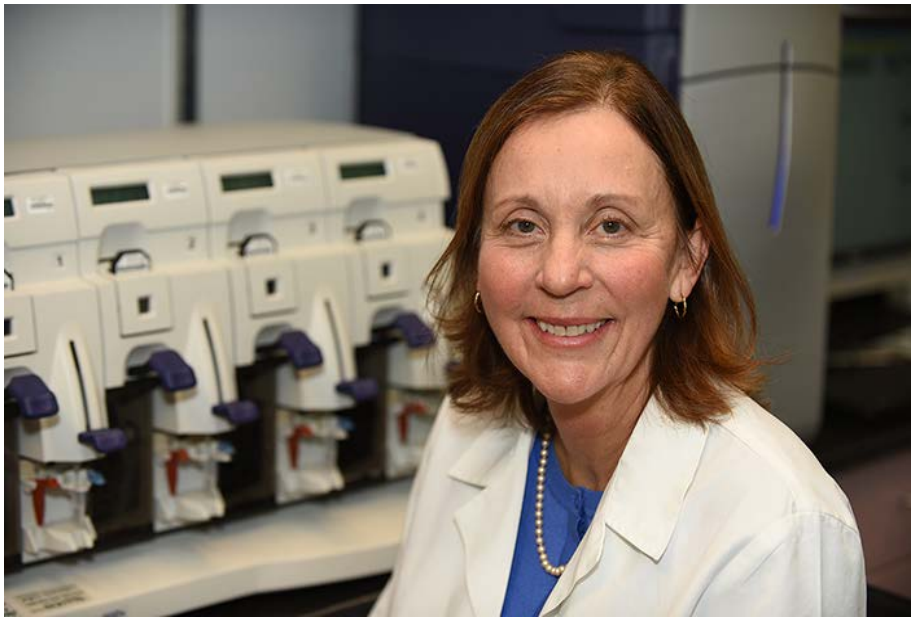

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Pranil Chandra, DO, FCAP

- **Chief Medical Officer, Genomic and Clinical Pathology and Services at PathGroup in Nashville, TN**
- **Fellowships in Oncological Surgical Pathology (Chief Fellow) and Molecular Genetic Pathology at UT MD Anderson Cancer Center**
- **Board Certified in Molecular Genetic Pathology, Anatomic & Clinical Pathology and Hematopathology**
- **Member of the CAP's PHC Committee**

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Abhijit A. Patel, MD, PhD

- **Associate Professor of Radiation Oncology at Yale University**
- **Residency training at Harvard; internship at MSKCC; MD/PhD at Yale**
- **Expertise in molecular biochemistry, genomics, and next-generation sequencing**
- **Research group at Yale is developing and validating ultrasensitive NGS-based assays for measuring circulating tumor DNA and RNA.**

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Disclosures

- **Dr. Chandra**
  - **Ownership interest in PathGroup.**
  - **Speaker/consultant for Pfizer, Roche, Bristol Myers Squibb, and Astra Zeneca.**
- **Dr. Patel**
  - **IP: Inventor on patent applications related to ctDNA assay technology.**
  - **Advisory roles: Novartis, NuGEN Technologies.**
  - **Research Funding: AstraZeneca**

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Agenda

- **Background on molecular principles and next generation sequencing**
- **Clinical utility and guiding appropriate testing utilization**
- **Background and emerging clinical applications for “liquid biopsy” testing**

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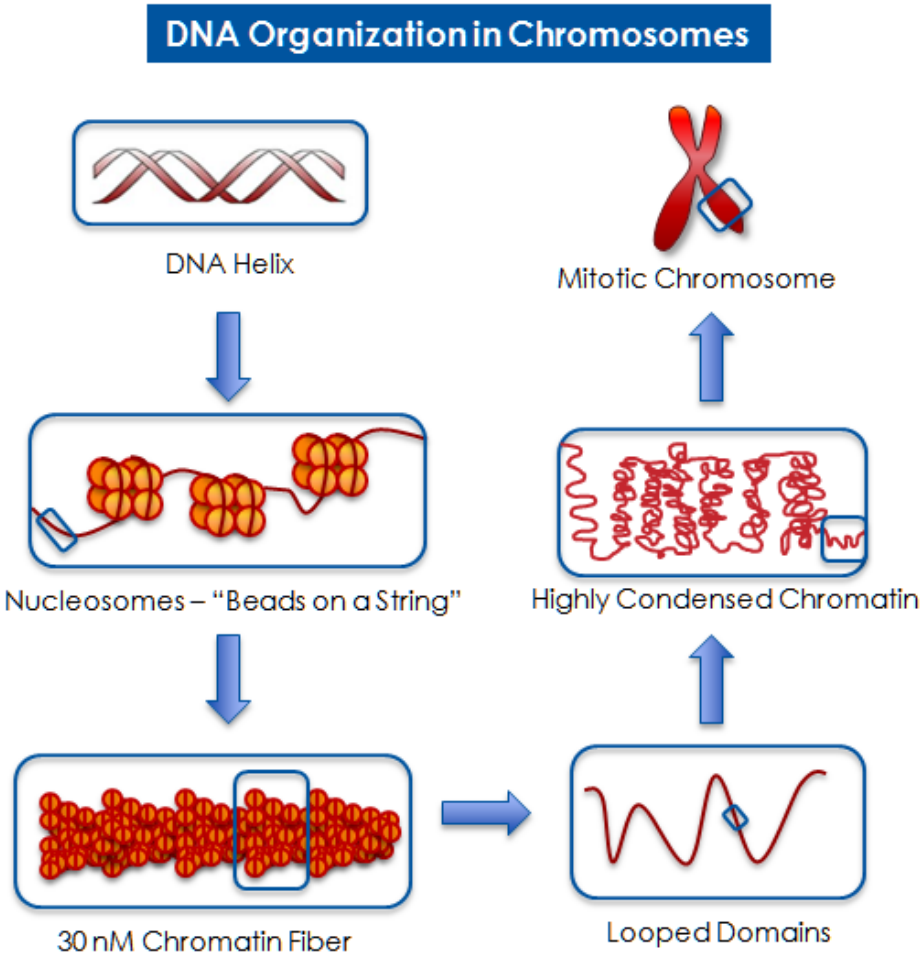
The Human genome is Composed of DNA…

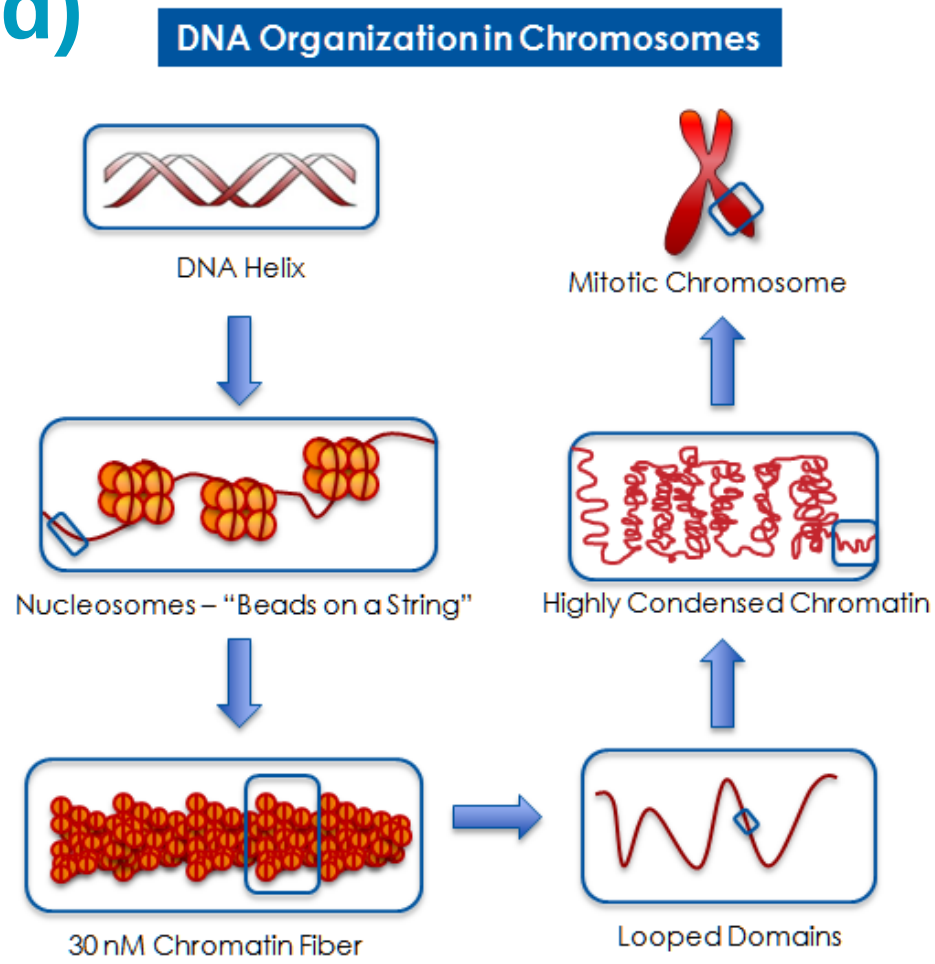
- **Total DNA in each cell of the human body:**
  - **Double stranded helix**
  - **Histones**
  - **Nucleosome**
  - **Chromatin fiber**
  - **Chromosome**



Source: DNA and Chromosomes. Penn State University Web site. https://wikispaces.psu.edu/display/230/DNA+and+Chromosomes. Updated August 10, 2009. Accessed November 4, 2014.

The Human genome is Composed of DNA… (continued)

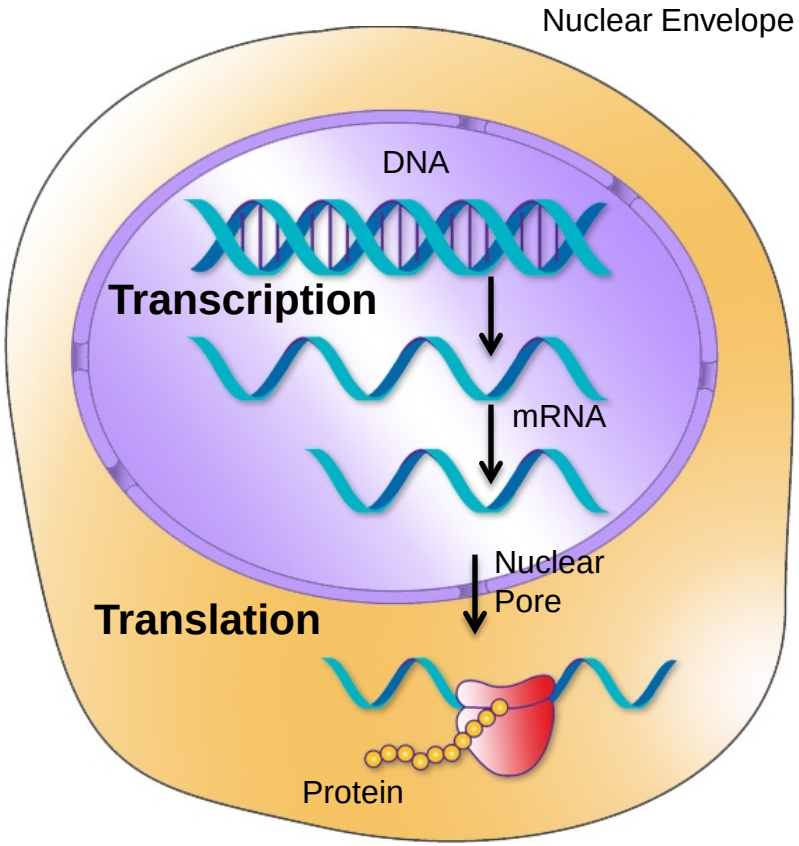
- **46 chromosomes (23 pairs)**
  - **Approximately 25,000 genes**
  - **Approximately 3 billion DNA base pairs**



Source: DNA and Chromosomes. Penn State University Web site. https://wikispaces.psu.edu/display/230/DNA+and+Chromosomes. Updated August 10, 2009. Accessed November 4, 2014.

…Which Directs the Production of Protein

- DNA codes for protein which directs functions of the body
  - Transcription
    - mRNA
  - Translation
    - Proteins involved in molecular pathways
      - Oncoproteins
      - Tumor Suppressor proteins



Source: Austin Peay State University Web site. http://apbrwww5.apsu.edu/thompsonj/Anatomy%20&%20Physiology/2010/2010%20Exam%20Reviews/Exam%201%20Review/Ch03%20The%20Nucleus.htm. Accessed November 4, 2014.

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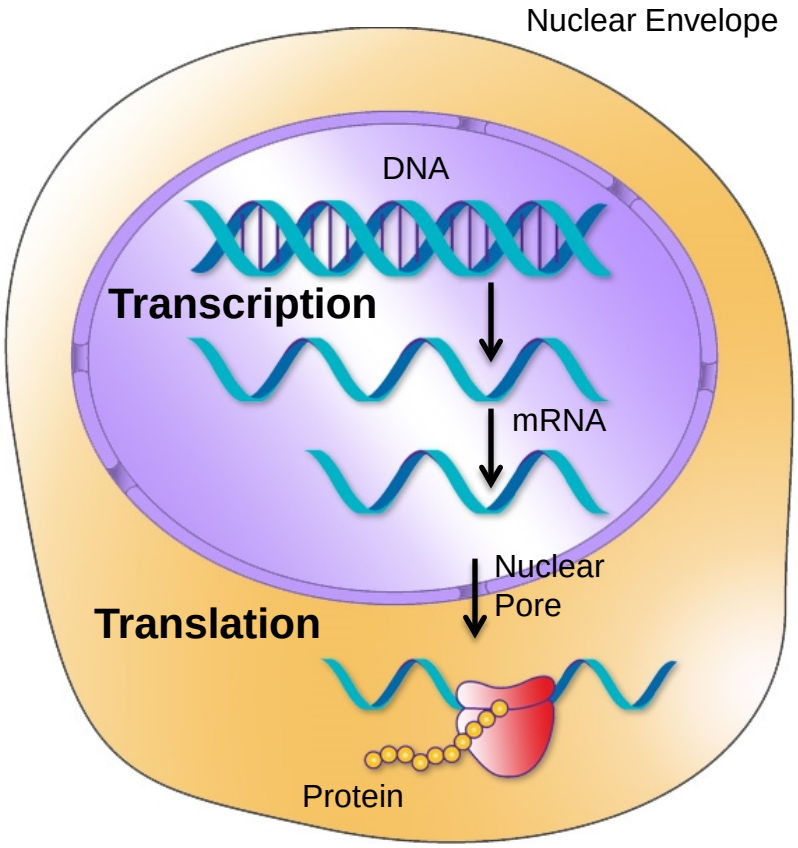
…Which Directs the Production of Protein (continued)

- **Molecular/genomic testing**
  - **Genetic**
    - **DNA, RNA, and/or protein**
  - **Epigenetic**
    - **Methylation**
    - **MicroRNA**



Source: Austin Peay State University Web site. http://apbrwww5.apsu.edu/thompsonj/Anatomy%20&%20Physiology/2010/2010%20Exam%20Reviews/Exam%201%20Review/Ch03%20The%20Nucleus.htm. Accessed November 4, 2014.

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Reasons For Growth of Cancer Genomics

- **Explosion of literature**
  - Identification of genomic aberrations that have clinical significance
    - Numerous genomic research efforts such as Cancer Genome Atlas Project (TCGA) and others
- **Marked increase in therapeutic drug development**
  - Greater than 500 targeted/cancer therapies in development over the next few years

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Reasons For Growth of Cancer Genomics (continued)

- **Marked decrease in costs to identify genomic aberrations**
  - Exponential decrease in sequencing costs
    - Rapid adoption of massively parallel (NGS) and other high throughput technologies

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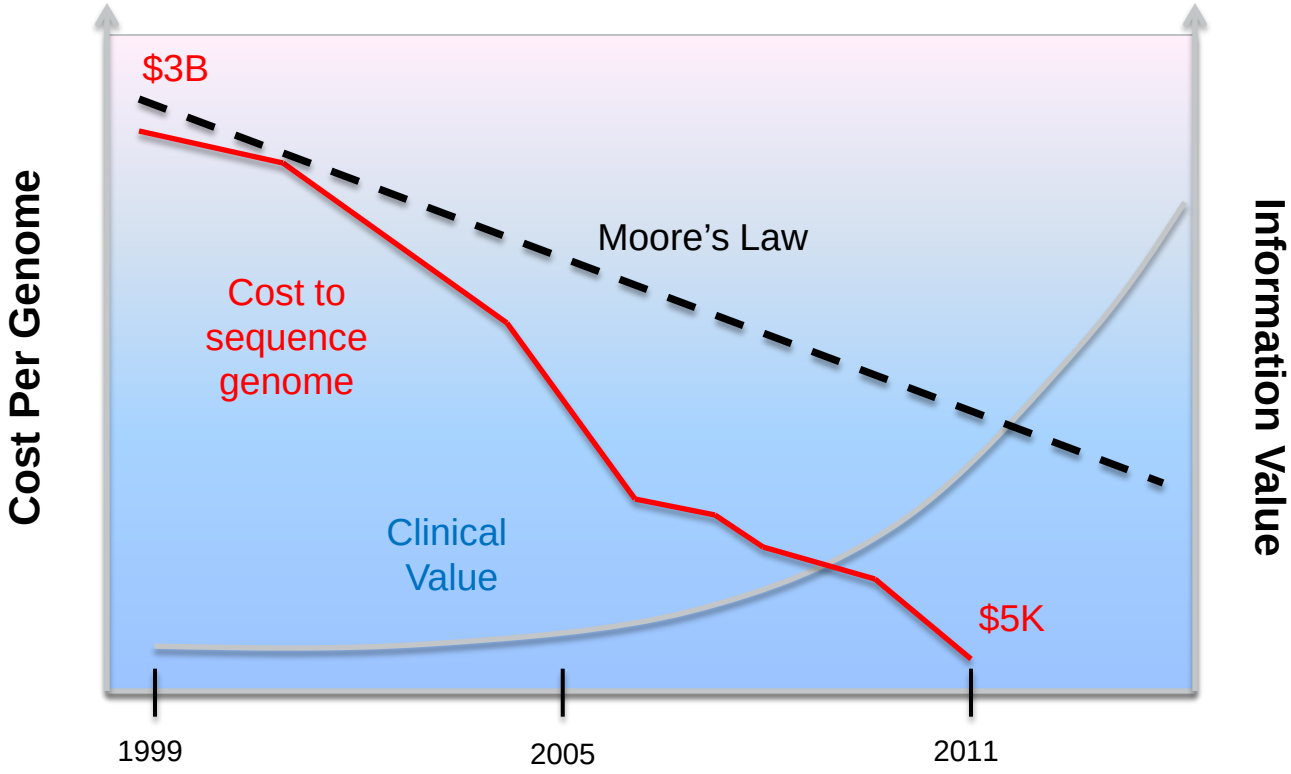
Opportunity – Actionable Information



Source: Andrews R. The era of genomic medicine…what to expect. Executive War College Web site. http://www.executivewarcollege.com/wp-content/uploads/2012/05/Andrews_for_Web3.pdf. May 2, 2012. Accessed October 17, 2014.

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Next-generation sequencing (NGS)

- **Basic principles**
  - **Disruptive technologies that allow for high throughput DNA/RNA sequencing**
    - **Assess for alterations in multiple (up to hundreds) of genes at once**
  - **Also known as massively parallel sequencing**

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Next-generation sequencing (NGS)

- **Advantages**
  - **More sensitive and accurate than Sanger Sequencing**
    - **Each section of DNA/RNA is sequenced multiple times.**
      - **Allows for greater depth of coverage (i.e. 500-1000X in oncology samples)**
    - **Can pick up mutations at low percentage compared with Sanger sequencing.**
      - **Advantageous in situations of molecular/tumor heterogeneity**

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Next-generation sequencing (NGS) (continued)

- **Advantages**
  - **Cost-efficient utilization of limited tissue samples to yield actionable information**
    - **Needs less DNA**
    - **Can test more than one sample at a time**
    - **Faster TAT compared to sequential testing**
  - **Information on multiple genes at once**
  - **Can process multiple patient samples at once**
- **Disadvantages**
  - **Expensive instrumentation and increased expertise required**
  - **Increased risk of contamination**
    - **Requires robust and standardized pre-analytic processes**
      - **Fixation, tissue processing, nucleic acid extraction, and library preparation**

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What is Clinical Utility?

- **Payer centric definition**
  - Improve patient outcomes
  - Decrease health care costs

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New Report

- **Joseph L, Cankovic M, Caughron S, et al. The Spectrum of Clinical Utilities in Molecular Pathology Testing Procedures for Inherited Conditions and Cancer: A Report of the Association for Molecular Pathology. *The Journal of Molecular Diagnostics*. 2016: 18(5): 605-619. doi: 10.1016/j.jmoldx.2016.05.007.**

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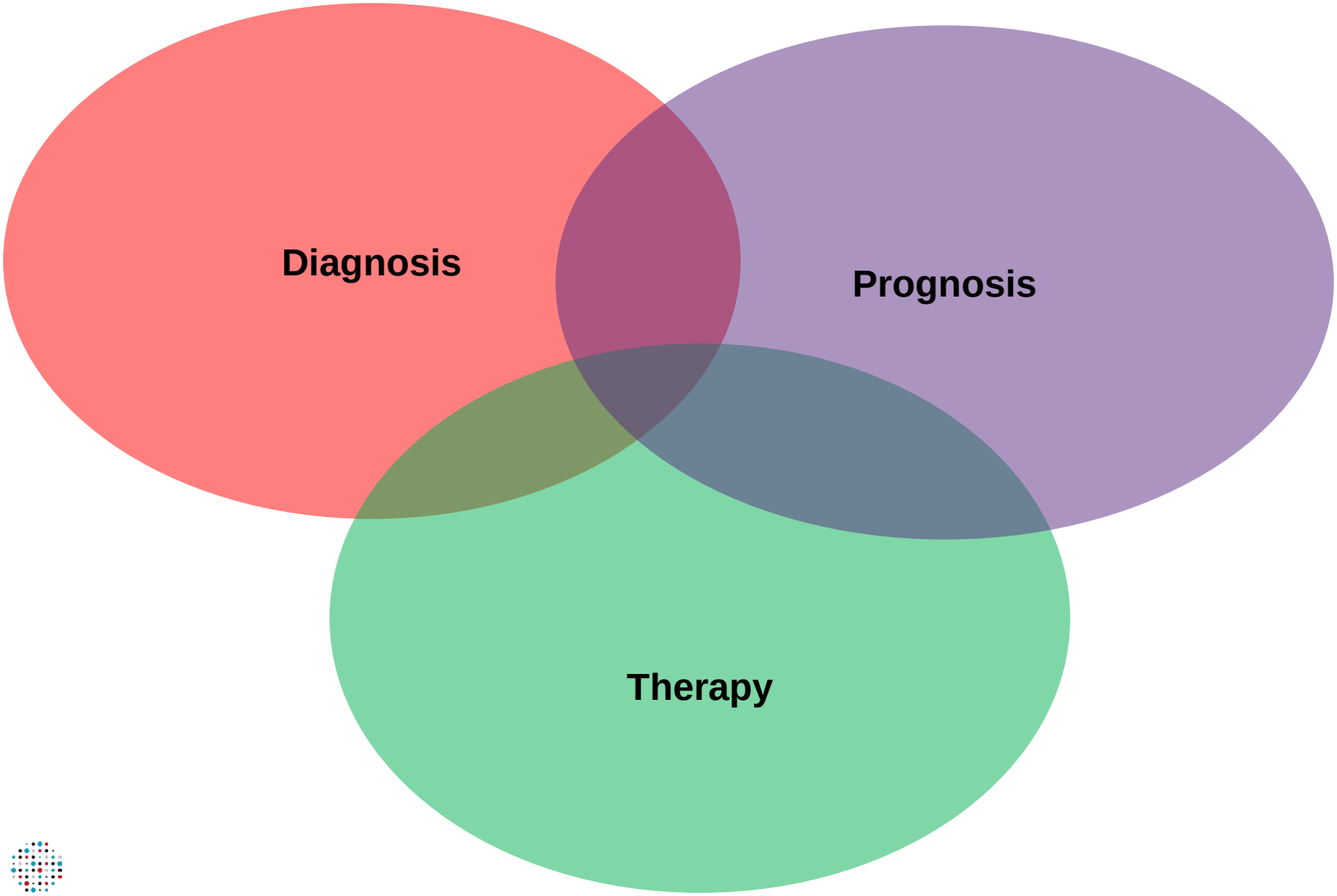
Precise Definition of Clinical Utility

- **Provide valuable information to the pathologist, treating oncologist, or other clinician—patient centric**
- **Alter clinical management decisions**

Diagnostic	• **Hematologic malignancies** o MDS, MPN, and MDS/MPN, end the "diagnostic odyssey". • **Poorly differentiated metastases**
Prognostic	• **Alter clinical management of patient** o Heightened vigilance o Institute therapeutic decisions earlier
Therapeutic	• **On or "off label" use of FDA approved targeted therapy** • **Investigational agent in a clinical trial setting** o **Greater than 500 targeted therapeutics** in clinical development over next 5 years

Clinical utility, patient-centric definition



Guiding Appropriate Utilization is Complex and Requires Pathology Direction

- **Diagnosis +/- Metastatic Disease**
  - Single analyte tests
  - Moving towards focused panel based testing with higher throughput technologies such as NGS
    - Especially in hematologic malignancies
- **Advanced/relapsed/refractory disease**
  - Higher throughput technologies such as NGS and broader interrogation are helping to guide scientifically driven clinical decision making
    - Targeted therapy in a clinical trial context

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Future of Cancer Genomics Testing

Single Analyte Tests→ Targeted NGS and/ or Multiplex Panel(s)

Performed on Tissue and/or Peripheral Blood

Broader Genomics Panels

Performed on Tissue and/or Peripheral Blood

Diagnosis +/- metastasis → Relapse/refractory → Advanced

- Diagnosis
  - Prognosis
    - Chemotherapy
      - Targeted Therapy
        - Clinical Trials
          - Other Management Decisions

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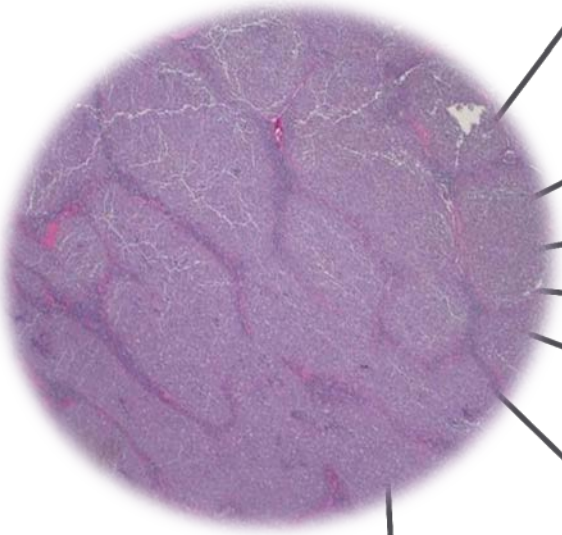
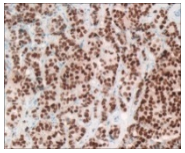
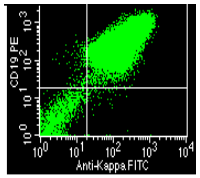
The Pathologist in the Era of Genomic Medicine



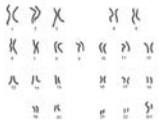
IHC +/-
Image Analysis



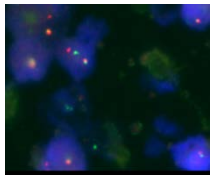
Flow cytometry



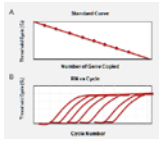
Cytogenetics and Cytogenomics



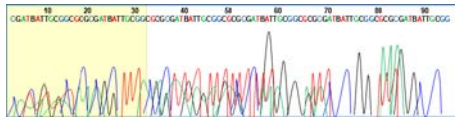
In-situ hybridization



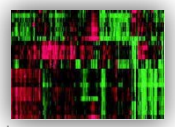
Polymerase chain reaction (PCR)



Sequencing
Sanger, Pyro, and NGS

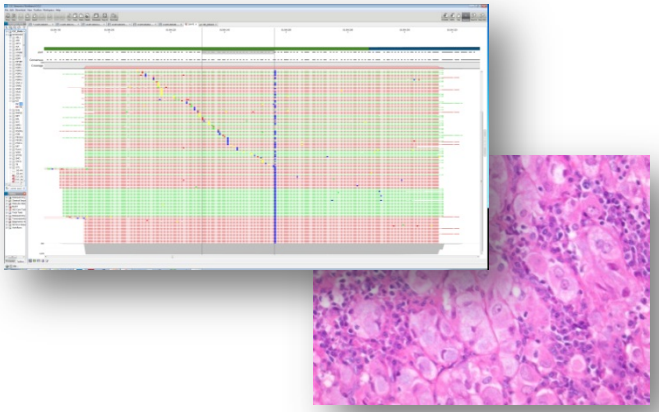


Gene Expression Profiling



Images courtesy of Pranil Chandra, DO: PathGroup

Integration of Morphologic, Genomic, and other Data



- Clinical findings
- Morphology
  - Quantitative analysis
- Genomic alterations
  - DNA
  - RNA
  - Protein

Molecular/ Genomic Testing

Clinical Informatics

- Analytic tools
- Genomic knowledgebase

- Diagnosis
- Prognosis
- Therapy
  - Clinical trials matching in advanced/refractory disease, as appropriate

Integrative reporting

Images courtesy of Pranil Chandra, DO: PathGroup

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“Liquid Biopsy”

- **Also known as:**
  - **ctDNA testing, cell-free DNA testing**
  - **Circulating tumor cell testing**
- **A plethora of commercial tests are available**
- **Numerous industry academic collaborations**
- **Important for pathologist to be involved in guiding test utilization and interpretation**

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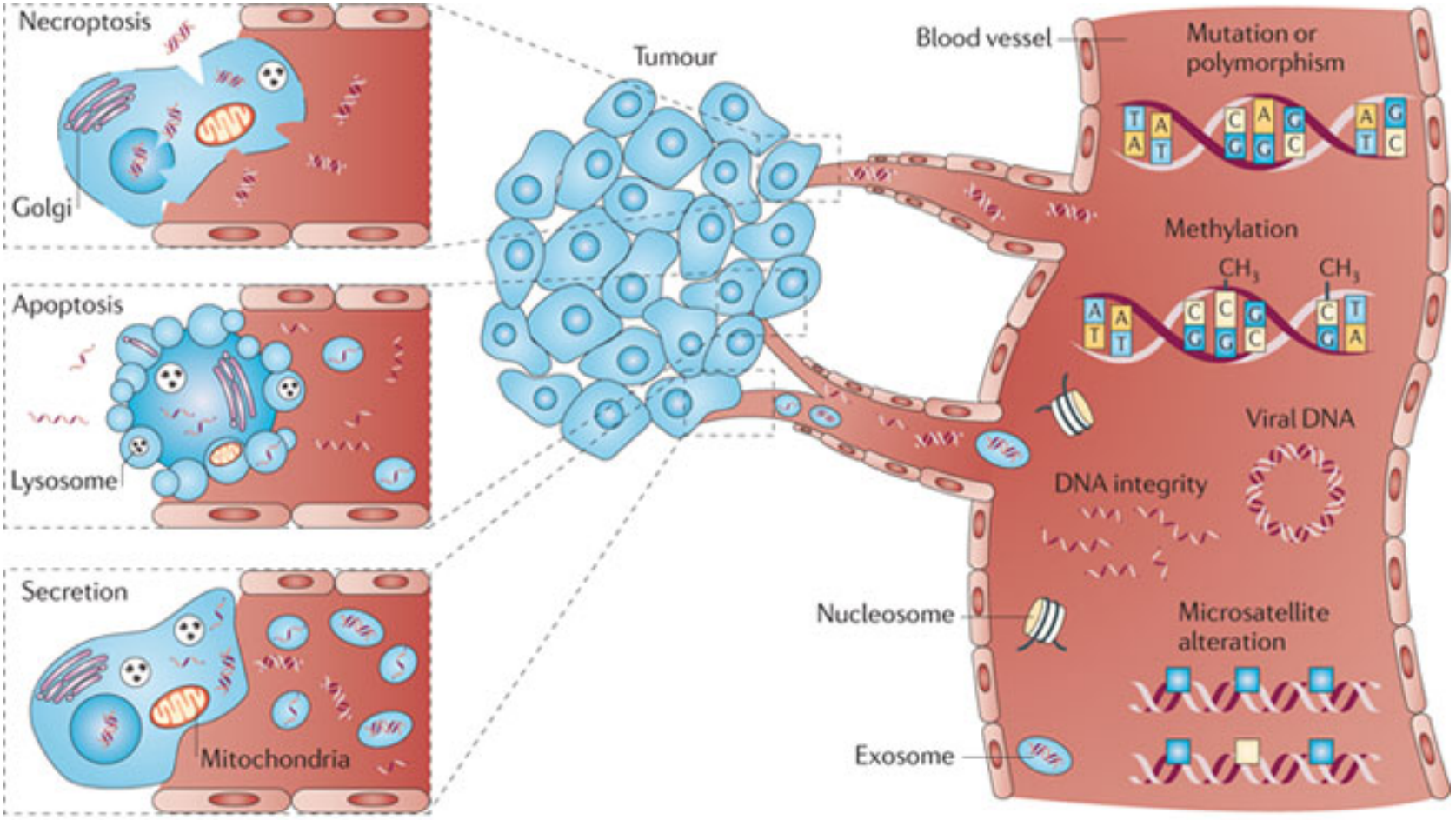
Various mechanisms of release of tumor DNA into peripheral blood



Source: Schwarzenbach H, et al, *Nature Reviews Cancer*. 2011; 11, 426-437

ctDNA: Summary of General Principles

- **Circulating tumor DNA (ctDNA) is shed from dying tumor cells into the peripheral blood.**
- **As a result, ctDNA can be detected in the peripheral blood or plasma of patients with various types of cancer.**

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ctDNA: Summary of General Principles (continued)

- **Presence of detectable ctDNA varies with type of disease and stage.**
  - There is a higher probability of detecting ctDNA in patients with advanced-stage malignancies.
  - Certain malignancies such as gliomas appear to shed less tumor DNA.

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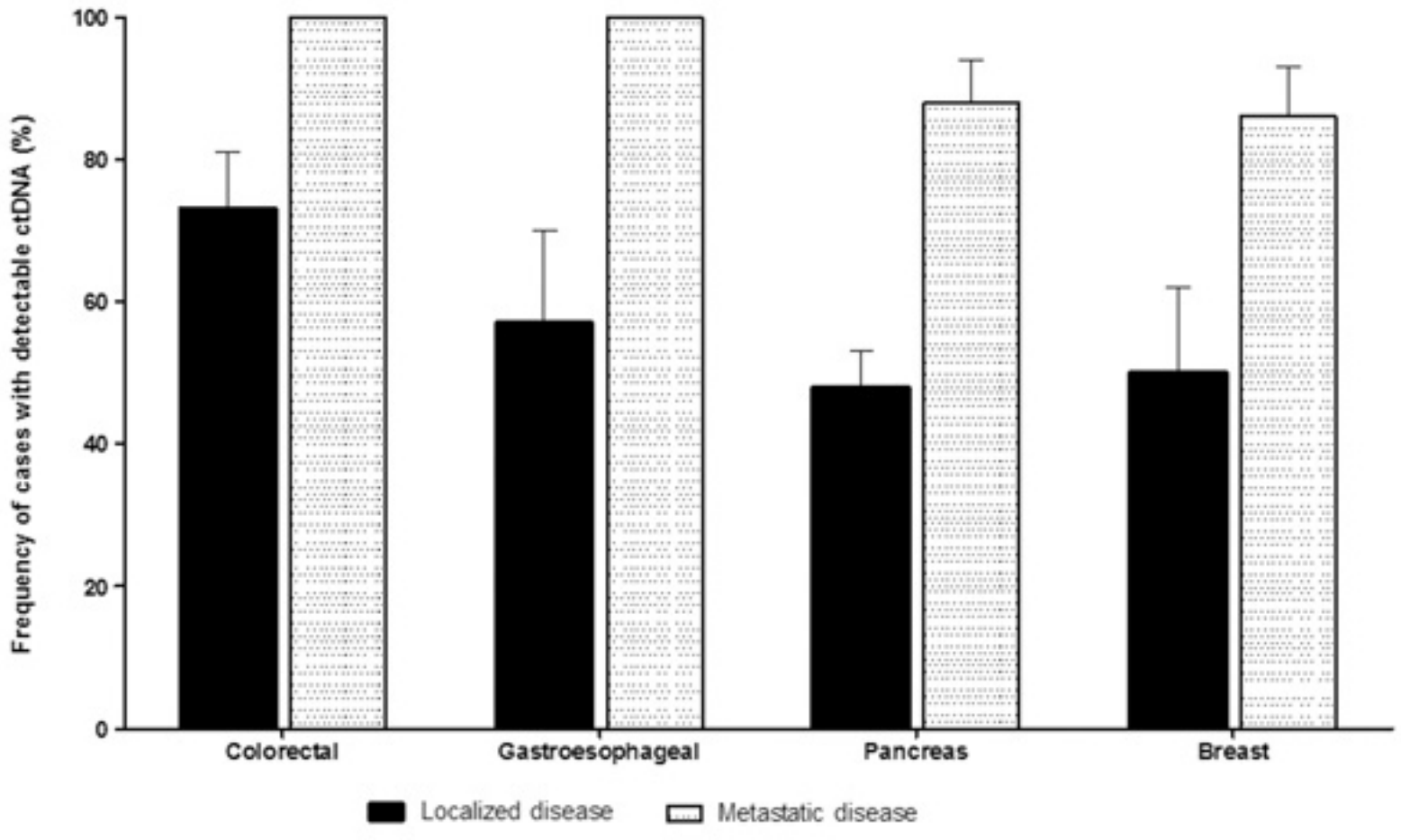
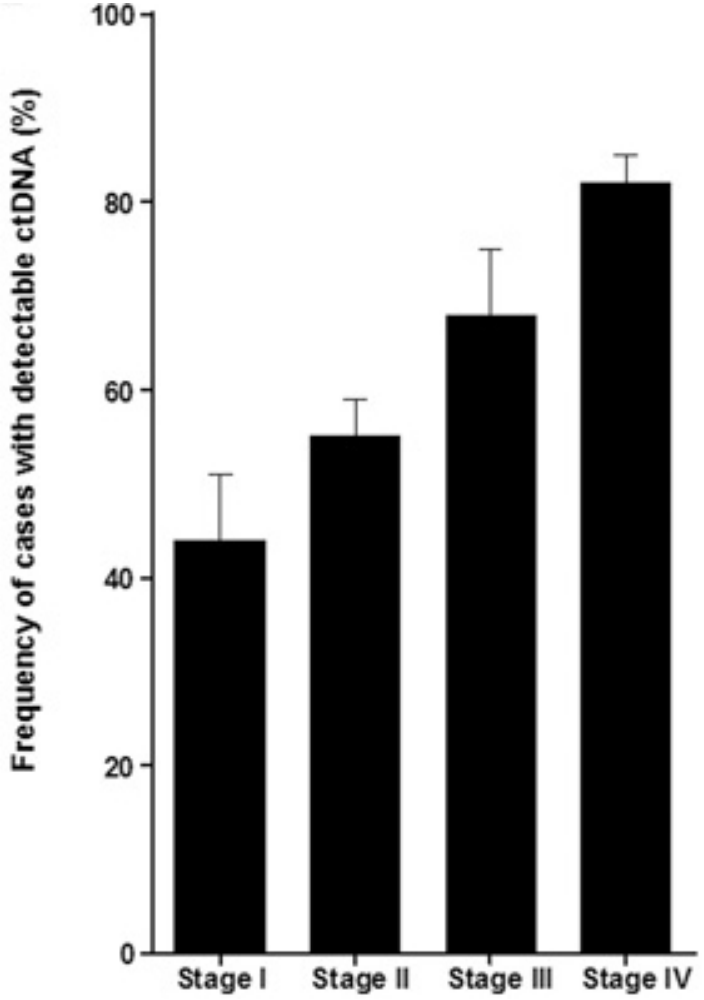
Detectability of Localized vs. Metastatic



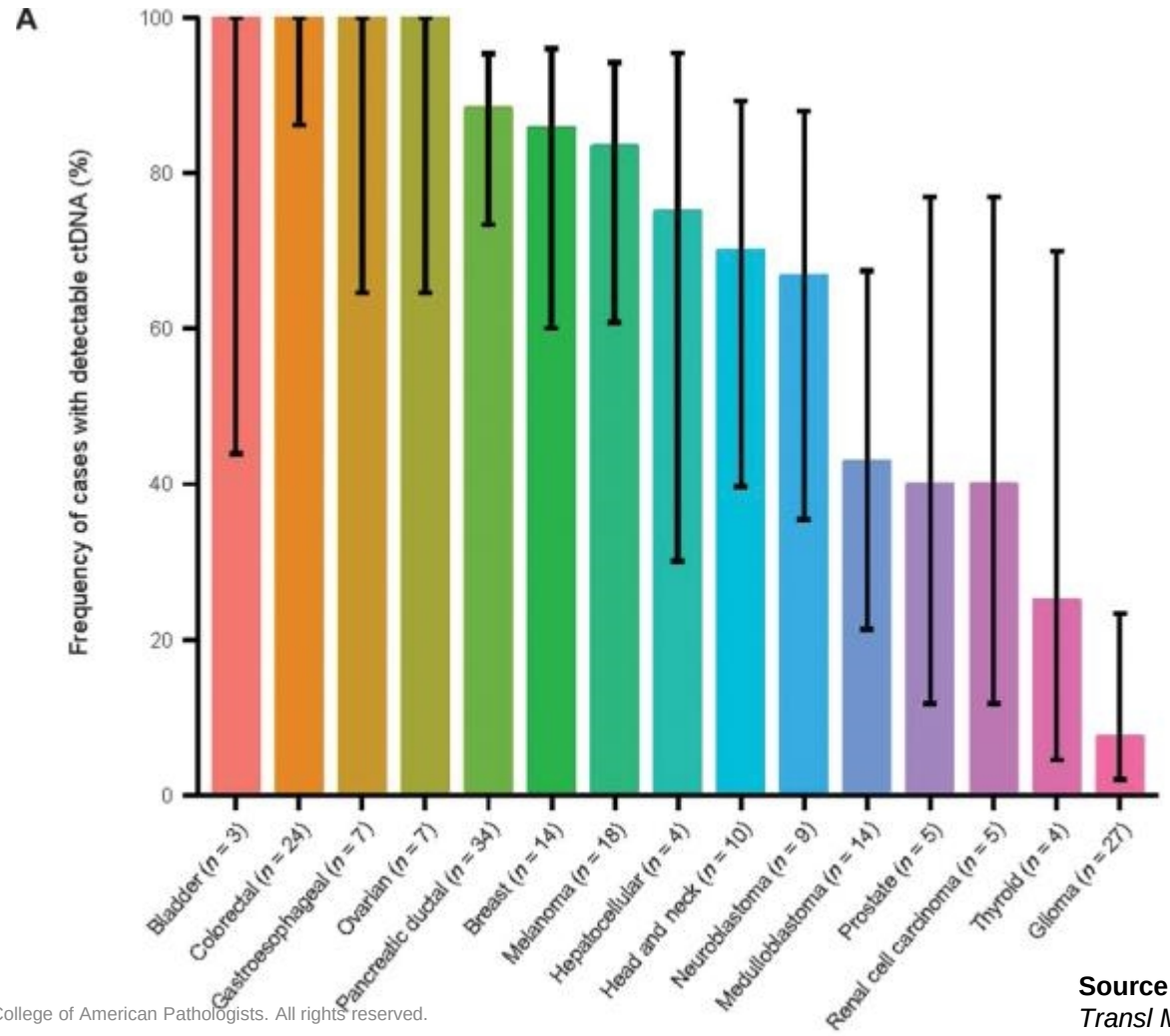
Source: Bettegowda C., et al., *Sci Transl Med.* 2014;6(224):224ra24

Circulating Tumor DNA (ctDNA) Detection Generally Varies by Stage



Source: Bettegowda C., et al., *Sci Transl Med.* 2014;6(224):224ra24

ctDNA Detection Can Vary by Cancer Type in Patients with Advanced Disease



Source: Bettegowda C., et al., *Sci Transl Med.* 2014;6(224):224ra24

ctDNA testing: Advantages and Disadvantages

- **Advantages**
  - Minimally invasive testing of blood samples may spare need for more invasive tissue biopsies.
  - Presence of mutation may have sufficient specificity to guide management decisions, in the appropriate context.
    - i.e. EGFR exon 19 deletion in NSCLC
  - Liquid biopsy may permit more comprehensive sampling of tumor mutation heterogeneity.
  - Plasma samples are generally processed more rapidly, which may reduce turn around time

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ctDNA testing: Advantages and Disadvantages (continued)

- **Disadvantages**
  - Pitfalls in inappropriate utilization of testing and/or misinterpretation of results.
    - Results require interpretation in appropriate clinical context.
    - Lower sensitivity, which confers higher false negative rate.
  - Low mutation abundance presents analytical challenges.

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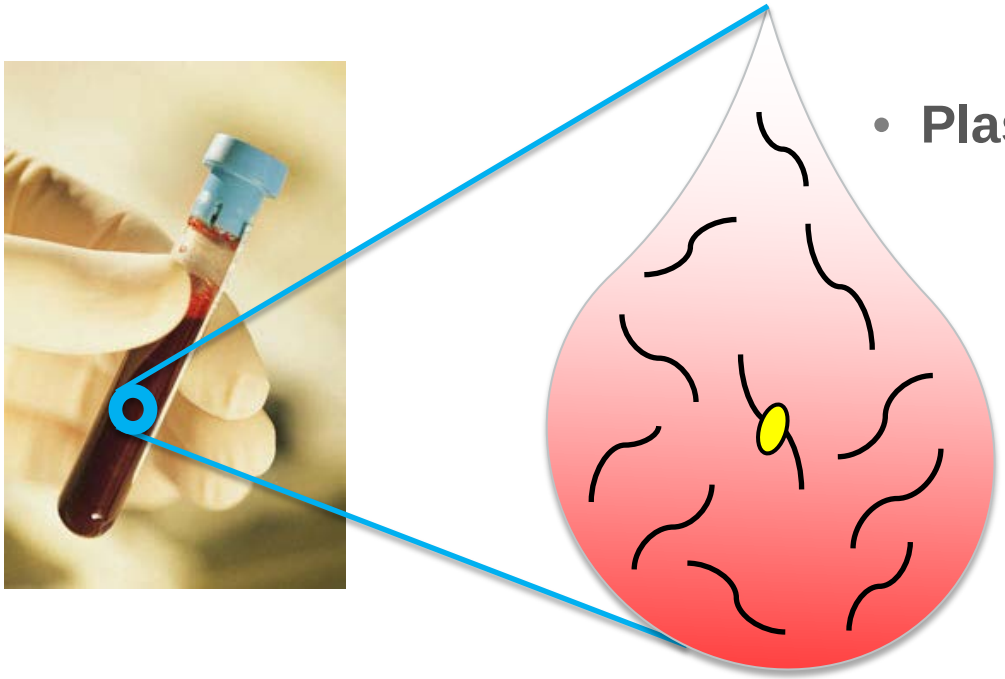
Challenges in Measuring ctDNA



- **Mutant tumor DNA is rare**
- **Excess background normal DNA**
- **Plasma DNA is fragmented**

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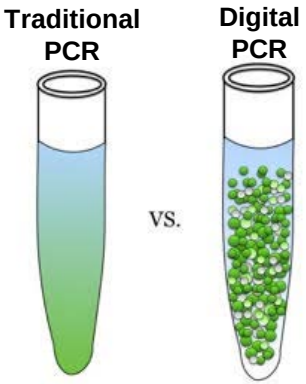
Common ctDNA assay strategies

- **Digital PCR**
  - Very sensitive detection of specific mutations of interest.
    - Limited to a small number of specific mutations per assay.
  - Need to know, clinically, what you are looking for
    - e.g. EGFR T790M mutation



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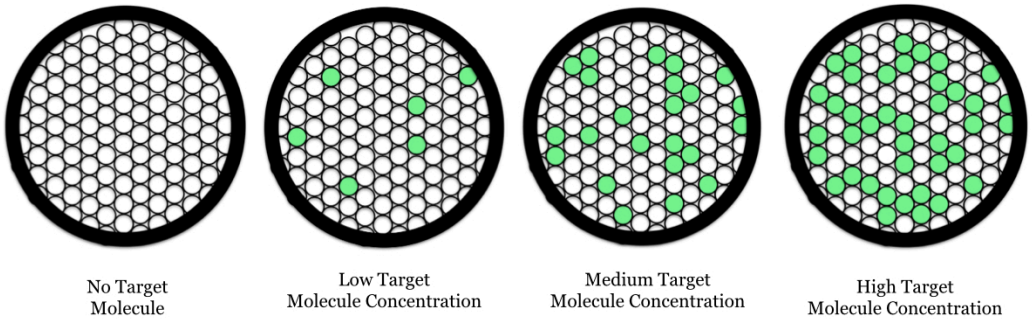

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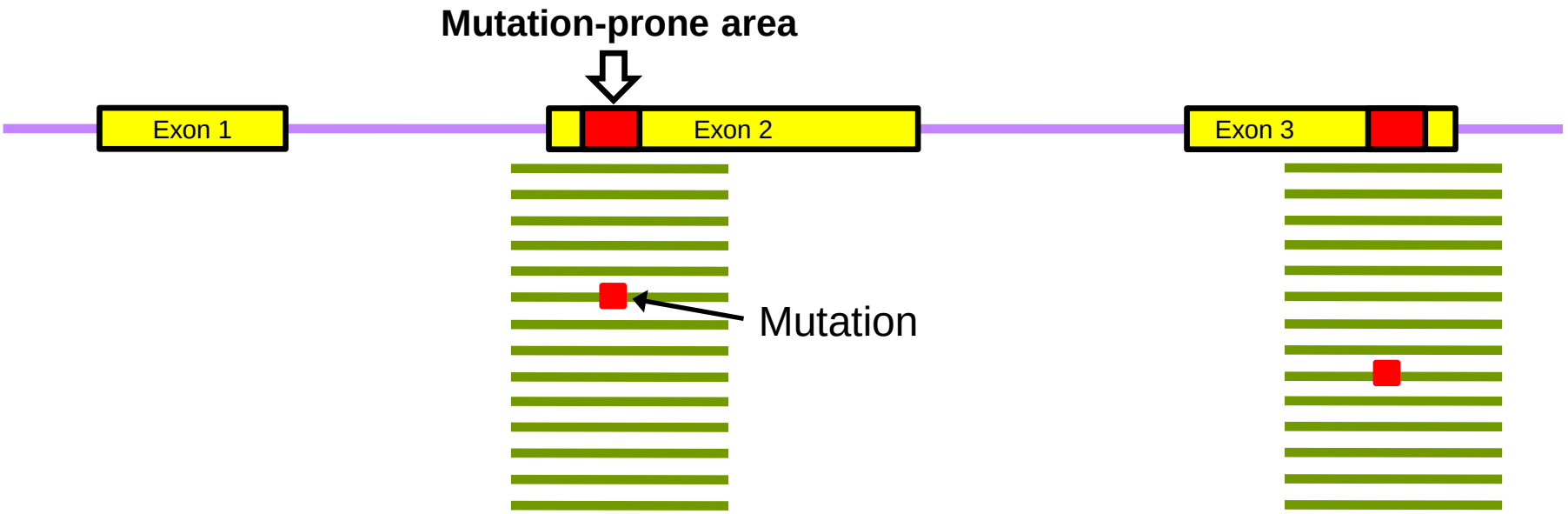
Common ctDNA assay strategies

- **Next generation sequencing**
  - Allows for broad interrogation of many different genes and types of alterations.
  - Comparable detection sensitivity as digital PCR (with new error suppression methods).

Common ctDNA assay strategies

- **Next generation sequencing**
  - Can be more expensive and has longer turn-around time.



Clinical applications of ctDNA

- **Current clinical applications**
  - Non-invasive genotyping of tumors to guide therapy.
    - e.g. EGFR mutation status in NSCLC to determine eligibility for EGFR tyrosine kinase inhibitors.
  - If negative, molecular testing of tissue biopsy is recommended.

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Clinical applications of ctDNA

- **Emerging clinical applications**
  - Monitoring for emergence of resistance mutations
  - Assessing treatment response
  - Residual disease monitoring
  - Detection of disease recurrence
- **Potential future application**
  - Early detection / cancer screening

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Areas of future development

- **Continue to monitor publications which will likely expand the clinical indications for ctDNA testing.**

- **Ongoing areas of investigation:**
  - Standardization of pre-analytic processes
  - Factors affecting ctDNA detection rate
  - Further improvements in assay technologies
  - Studies to establish clinical utility of ctDNA in various settings

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Summary

- **ctDNA-based mutation profiling may be utilized in specific and limited clinical contexts such as NSCLC.**
- **Numerous studies are ongoing to explore broader areas of clinical utility and standardization.**
- **Clinical applications for ctDNA-based testing will continue to grow.**

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DATE	TOPIC	SPEAKER(s)
Archived	HER2 Testing and Clinical Decision-making in Gastroesophageal Adenocarcinoma	Mary Kay Washington and Jaffer Ajani
Archived	Molecular Biomarkers for the Evaluation of Colorectal Cancer: New evidence-based guideline from ASCP, CAP, AMP and ASCO	Antonia Sepulveda
Archived	The Cancer Protocols and Changes in Tumor Staging	Thomas Baker

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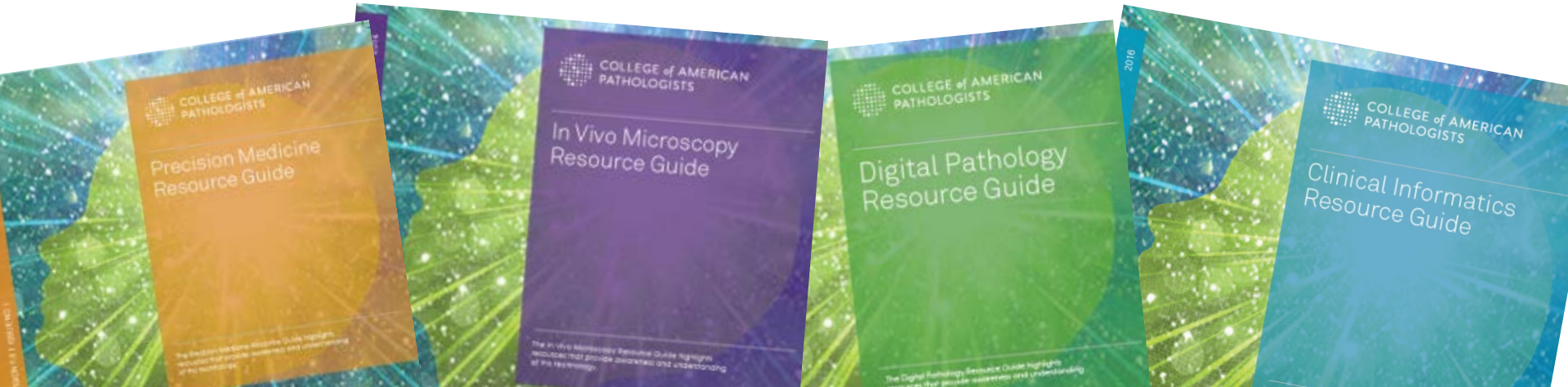
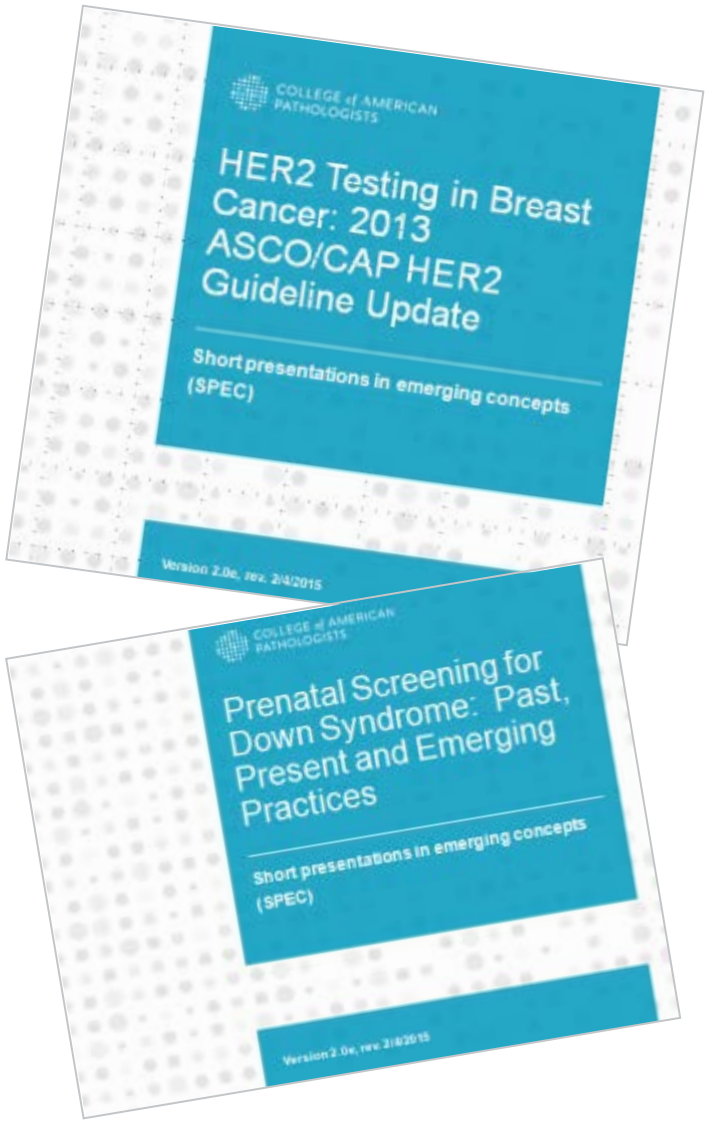
- **The CAP has created the Pathology Resource Guides to assist pathologists in understanding key emerging technologies.**
  - **Printed guides are now available for members ($39) and non-members ($69)**
  - **The digital copy of the Resource Guides are a complimentary member benefit**
  - **Access them www.cap.org > Resources and Publications**



Short Presentations on Emerging Concepts (SPECS)

- **Pathology SPECs are:**
  - **short PowerPoints, created for pathologists**
  - **Focused on diseases where molecular tests play a key role in patient management**
- **Topics include Renal Tumors, cell free DNA (cfDNA), and PD-L1 as well as other emerging topics**
- **Access them www.cap.org > Resources and Publications**



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